THE VARIATION OF SURFACE TENSION OF SOLUTIONS OF SODIUM HEXADECYL SULPHATE WITH TIME

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Summary

The rate of fall of surface tension at the surfaces of solutions of sodium hexadecyl sulphate is shown to be greatly influenced by traces of electrolyte present even in treble distilled water. The rate of fall could be shown to be governed by diffusion through a convecting system, hexadecanol being used as a tracer.

Solutions of Na^+ (1×10^{-3}M), Ca^+ (1×10^{-6}M), Mg^{++} (1×10^{-6}M), or Fe^{++} ($1\cdot8\times10^{-7}$M) produced rapid changes in surface tension. In the presence of a large excess of NaCl the rate of fall of surface tension became independent of trace impurities (at least for periods up to 4 hr) and this rate was approximately governed by diffusion of hexadecyl sulphate ion to the surface.

The experiments could not detect any electrical energy barrier to adsorption.

I. Introduction

The surface tensions of dilute solutions of paraffin chain salts have been observed (cf. Adam and Shute 1938) to decrease over many hours. These changes have been attributed (e.g. Doss 1936*a*, 1936*b*, 1938*a*, 1938*b*, 1939, 1947) to a potential barrier induced by charged ions at a surface, or by Alexander (1941) to reorientation of the surface-active species in the surface. Some of the slow decrease may also be due to small amounts of impurity in the paraffin chain salt as these are notoriously difficult to purify. The impurities may be homologues of the salt, other electrolytes, or the organic starting material used to prepare the salt. Recent results of Haydon and Phillips (1956, 1958) have emphasized the importance of trace electrolytes on the equilibrium interfacial tensions of paraffin chain salts and in particular the extreme sensitivity of the alkyl sulphates.

The rate at which the surface tension falls is considerably slower than corresponds to the rate of diffusion of the paraffin chain salt to the surface. Because the addition of any electrolyte considerably increases the rate, it is generally held that adsorption in the absence of electrolyte is hindered by an energy barrier which is decreased by additional electrolyte.

Careful measurements on solutions of sodium hexadecyl sulphate were made by Nutting, Long, and Harkins (1940) and this substance was therefore chosen

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for study. Nutting, Long, and Harkins (1940) found at 40 °C that solutions containing less than 6×10^{-4}M per litre, the critical micelle concentration (c.m.c.), reached equilibrium slowly whereas those above this concentration equilibrated fairly rapidly. My object was to check their results and then to obtain a quantitative theory of the kinetics of adsorption. The results reported herein differed considerably from those of the above authors so that it became necessary to check the effects of a number of impurities as well as to derive new data on the effect of electrolytes.

II. Experimental Methods

Hexadecanol was fractionally distilled and the sulphate obtained by the method of Shedlovsky (1946). After extraction and crystallization as described by that author the samples were extracted with dry ether for 48 hr and then fractionally crystallized from ethanol.

The sodium content was $100 \cdot 3 \pm 0 \cdot 5$ per cent. of theoretical and using a modified method of Auerbach (1943) in which the alkyl sulphate is titrated against hexadecyl trimethylammonium bromide the purity appeared to be $99 \cdot 5 \pm 0 \cdot 5$ per cent.

A sensitive test for purity of a paraffin chain salt is given by the variation in equilibrium surface tensions with concentration which should exhibit no minimum near the c.m.c. Figure 2 shows that a minimum would appear in the surface tension concentration curve at times exceeding 30 min but judged on the difference between curves (*d*) and (*e*) at 200 min the sample used was as pure or purer than that used by other investigators (Powney and Addison 1937 ; Nutting, Long, and Harkins 1940).

The surface tension was measured using the vertical film balance of Harkins and Anderson (1937). The experimental arrangement was similar to that of Addison and Hutchinson (1948), the sensitivity being $0 \cdot 2$ dyne cm^{-1} with an absolute error of less than $0 \cdot 5$ dyne cm^{-1}. The plate was of mica (3 cm wide by $0 \cdot 0014$ cm thick) the surface of which had been abraded with fine emery paper to produce a mat surface (Matalon and Schulman 1949). This treatment produced an apparent receding angle of zero and enabled the plate to be used for long periods without cleaning. This is essential where experiments last for several hours. The surface tensions of water and some alcohol solutions were used to check the film balance. Surface tensions could be measured within 15 sec of generating a surface. Measurements were at 40 ± 1 °C in a closed thermostat, the atmosphere being rapidly saturated by using moistened sheets of filter paper.

The trough containing the solution was of polytetrafluoroethylene which was satisfactory in that it did not liberate either organic or inorganic impurities. Thus water was allowed to stand for 4 hr in the trough and the surface compressed to one-tenth of its initial area. There was no change in its surface tension ($\pm 0 \cdot 2$ dyne cm^{-1}).

Films of insoluble monolayers of hexadecanol were spread from solutions in purified light petroleum.

The salts used were of analytical quality. Because polyvalent ions are important, the percentage maximum impurities (from the labels) are recorded here :

	Pb	Fe	Ba	Ca	Mg
NaCl :	0·001	0·0005	0·003	0·005	0·005
$CaCl_2$:	0·002	0·001	—	—	0·02
$MgCl_2$:	0·001	0·0005	0·01	0·005	—

The water was distilled from neutral permanganate, and twice redistilled through a borosilicate apparatus using a large splash head to prevent carry-over of spray. The water was stored in a quartz vessel for not longer than 3 days but the " best " results were obtained using water prepared the same day as the experiment. Stock solutions of sodium hexadecyl sulphate (0·0005M) were prepared each day, the pH value varying from 6·6 to 6·8.

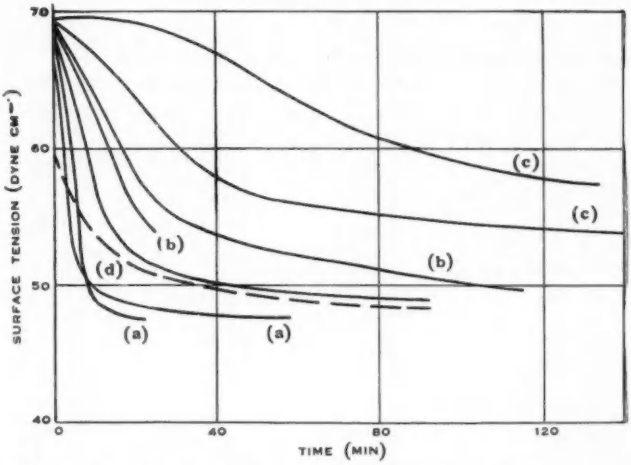

Fig. 1.—The variation of surface tension with time for a solution containing $5{\cdot}0\times10^{-5}$M sodium hexadecyl sulphate/l.

(*a*) Single distilled water ; (*b*) double distilled water ; (*c*) treble distilled water ; (*d*) data of Nutting, Long, and Harkins (1940) using conductivity water. Surface tension of water at 40 °C is 69·6 dyne cm^{-1}.

III. Results of Measurements

(*a*) *Effect of Purity of Water*

Figure 1 compares extreme results for solutions prepared with water distilled one, two, and three times as well as the results of Nutting, Long, and Harkins (1940), who used conductivity water. The effect of purity of water is quite dominant and suggests that previous published results are seriously in error. The slowest fall in surface tension is taken to represent the purest sample of water but it is clear that the curves, even with water distilled three times, are

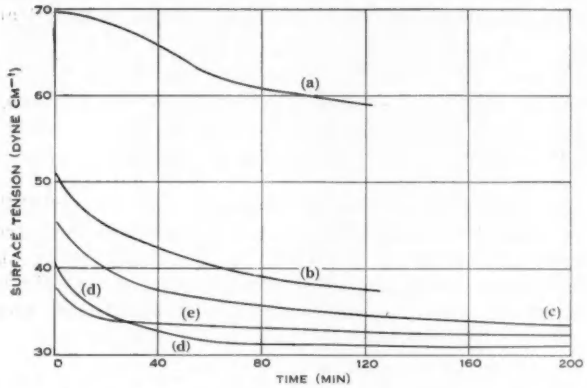

Fig. 2.—The variation of surface tension with time for solutions of sodium hexadecyl sulphate in treble distilled water.
(*a*) 5×10^{-5}; (*b*) 3×10^{-4}; (*c*) 4×10^{-4}; (*d*) 5×10^{-4}; (*e*) 1×10^{-3}.

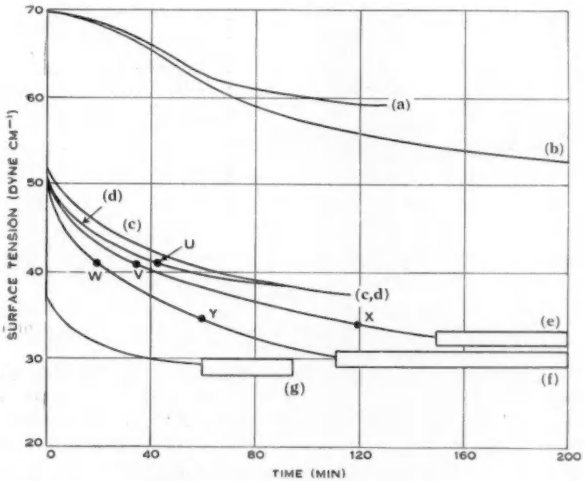

Fig. 3.—The effect of added hexadecanol on the variation of surface tension with time of solutions of sodium hexadecyl sulphate.

$C_{16}H_{33}SO_4Na = 5{\cdot}0\times10^{-5}$M/l: (*a*) No added hexadecanol; (*b*) hexadecanol : hexadecyl sulphate weight ratio = 0·01 : 1.

$C_{16}H_{33}SO_4Na = 3{\cdot}0\times10^{-4}$M/l: (*c*) No added hexadecanol; (*d*) weight ratio of alcohol to sulphate, 0·01 : 1; (*e*) weight ratio of alcohol to sulphate, 0·02 : 1; (*f*) weight ratio of alcohol to sulphate, 0·05 : 1; (*g*) as for (*e*) but with NaCl added to raise Na^+ to 10^{-3}M/l.

highly variable. For this reason each set of results presented in each of the various figures was obtained on one sample of water. Unless specifically mentioned the water used in preparing solutions was distilled three times.

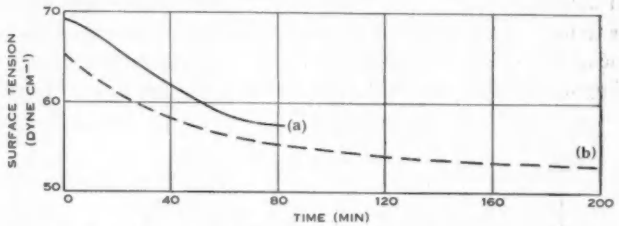

Fig. 4.—The effect of a monolayer spread on the solution after sweeping surface.

Sodium hexadecyl sulphate $5{\cdot}0\times10^{-5}$M/l: (*a*) No hexadecanol; (*b*) hexadecanol at an area of 50 Å² per molecule.

(*b*) *Variation in Concentration of Hexadecyl Sulphate*

At higher concentrations the purity of the water is less important and the curves become readily reproducible (Fig. 2), even the difference from the results

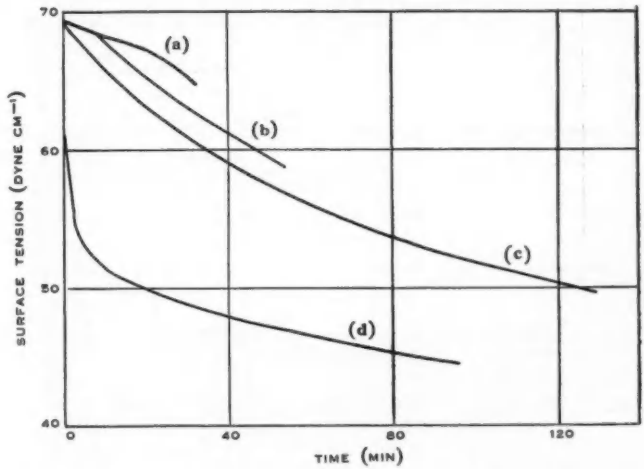

Fig. 5.—The effect of added sodium octadecyl sulphate on the fall of surface tension with time for a solution containing 5×10^{-5}M sodium hexadecyl sulphate/l.

(*a*) No added octadecyl sulphate; (*b*) weight ratio of octadecyl to hexadecyl sulphate, 0·01 : 1; (*c*) weight ratio of octadecyl to hexadecyl sulphate, 0·05 : 1; (*d*) as for (*c*) but with added NaCl to make Na^+ concentration to 10^{-3}M/l.

of Nutting, Long, and Harkins being small. The intersection of curves (*d*) and (*e*) indicates the presence of impurities, the effect of which is negligible as soon as the c.m.c. (6×10^{-4}M) is exceeded.

(c) Effect of Hexadecanol and Sodium Octadecyl Sulphate

Both these substances are usually present in purified samples of sodium hexadecyl sulphate. Figure 3 indicates that the amount of hexadecanol in the hexadecyl sulphate must exceed 1 per cent. if any considerable change in the kinetics is to be apparent within 2 hr. For more than 1 per cent., the monolayer was plastic and the plate showed marked sticking in its movement, the measurements being reproducible only to ± 1 dyne cm^{-1} (indicated by open boxes). When

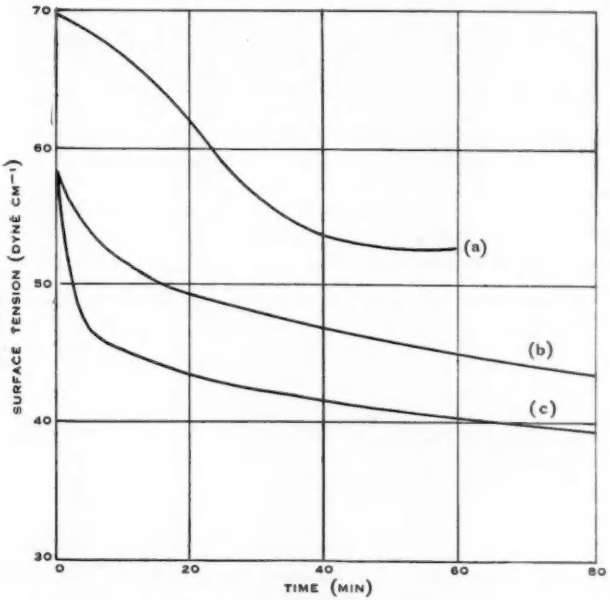

Fig. 6.—The effect of added hydrogen ion (10^{-3}M) on the fall of surface tension with time for a solution containing $5{\cdot}0\times10^{-5}$M sodium hexadecyl sulphate/l.

(*a*) No added H^+; (*b*) $H^+ = 1{\cdot}0\times10^{-3}$M/l, first run; (*c*) duplicate run commenced 90 min after preparing the solution.

this stage was reached, the surface tensions remained unchanged for at least 18 hr. A monolayer of hexadecanol was also spread on a freshly swept surface of a " pure " solution and the subsequent fall in surface tension recorded (Fig. 4). The effect of octadecyl sulphate (Fig. 5) is far less marked. This, therefore, is not a serious impurity for the times studied. Both these additives appear to be relatively unimportant impurities for times up to 2 hr, although for greater than 12 hr they may be dominant.

(*d*) *Effect of Inorganic Salts*

The addition of acid (Fig. 6) caused a rapid fall in surface tension but duplicates on the acid solution stored for 90 min showed that hydrolysis must

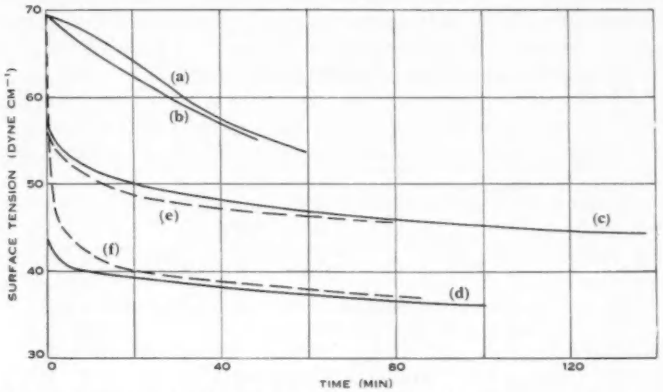

Fig. 7.—The effect of added sodium chloride on the fall of surface tension with time of a solution containing $5{\cdot}0\times10^{-5}$M sodium hexadecyl sulphate/l.
(*a*) No added sodium chloride; (*b*) NaCl$=1{\cdot}0\times10^{-4}$M/l; (*c*) NaCl$=1{\cdot}0\times10^{-3}$M/l; (*d*) NaCl$=5{\cdot}0\times10^{-3}$M/l; (*e*) NaCl$=1{\cdot}0\times10^{-2}$M/l. Single distilled water; (*f*) theoretical curve based on diffusion-governed mechanism in a convecting system.

have been considerable as curve (*c*) obtained at this time is markedly lower than freshly prepared solution (*b*).

Sodium chloride (Figs. 7, 8) caused a greatly increased rate of fall of surface tension and for concentrations greater than or equal to 10^{-3}M the purification of

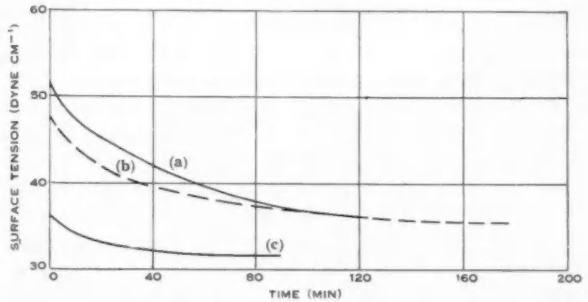

Fig. 8.—The effect of added ions on the fall of surface tension with time of a $3{\cdot}0\times10^{-4}$M sodium hexadecyl sulphate solution.
(*a*) No added eletrolyte; (*b*) $CaCl_2=1{\cdot}0\times10^{-6}$M/l; (*c*) $Na^+=1{\cdot}0\times10^{-3}$M/l.

the water used to prepare the solutions seemed unimportant. The surface tensions at any given time would repeat to within 0·2 dyne cm^{-1}.

Divalent ions such as calcium (Figs. 8, 9) and magnesium (Fig. 10) produced large effects at very low concentrations.

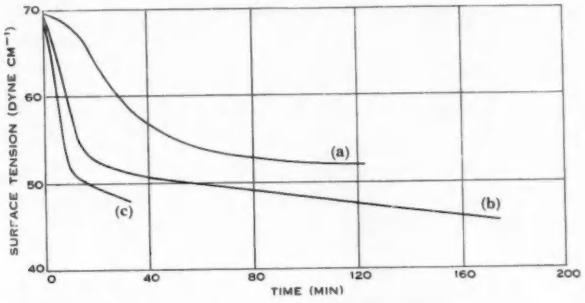

Fig. 9.—The effect of added calcium on the fall of surface tension with time of a solution containing $5{\cdot}0\times10^{-5}$M sodium hexadecyl sulphate/l.
(*a*) No added calcium and $CaCl_2=10^{-7}$M/l; (*b*) $CaCl_2=1{\cdot}0\times10^{-6}$M/l; (*c*) $CaCl_2=1{\cdot}0\times10^{-5}$M/l.

Ferric salt (Fig. 11) was even more effective than calcium or magnesium despite the hydrolysis which was evident in the stock iron solution (0·01M)

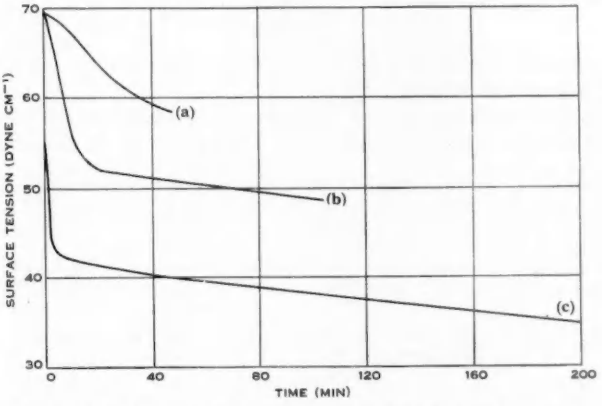

Fig. 10.—The effect of added magnesium on the fall of surface tension with time of a solution containing $5{\cdot}0\times10^{-5}$M sodium hexadecyl sulphate/l.
(*a*) No added magnesium; (*b*) $MgCl_2=1{\cdot}0\times10^{-6}$M/l; (*c*) $MgCl_2=1{\cdot}0\times10^{-5}$M/l.

which had a pH value of 6·0. It is unlikely that the results were due to hydrolysis of the hexadecyl sulphate solution as after injection of the iron solution the pH value was 6·4.

IV. Discussion

(a) *General*

The results taken as a whole suggest that the slow drifts of surface tension with time of the more dilute solutions are substantially due to impurities which are polyvalent electrolytes. These impurities must reach the surface to be effective, and the following mechanisms are examined. At one extreme there is a process controlled entirely by diffusion through a stationary body of liquid. At the other extreme transfer is governed only by an energy barrier at the surface. Intermediate cases arise where part of the solution is stirred by convection, the solute diffusing through a substantially immobile layer near the surface either with or without a further energy barrier hindering entry into the surface. A detailed discussion of such systems has been given elsewhere (Sutherland 1952).

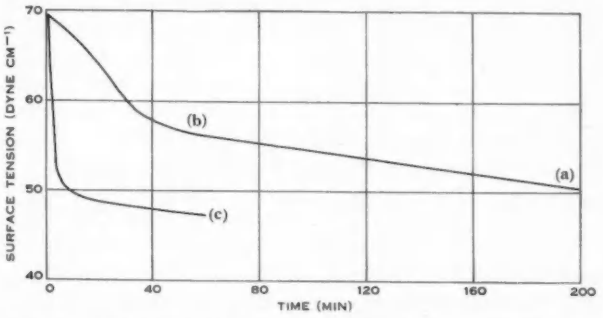

Fig. 11.—The effect of added iron on the fall of surface tension with time of a solution containing $5{\cdot}0\times10^{-5}$M sodium hexadecyl sulphate/l.

(*a*) No added iron; (*b*) $FeNH_4(SO_4)_2 12H_2O = 1{\cdot}3\times10^{-8}$M/l; (*c*) $FeNH_4(SO_4)_2 12H_2O = 1{\cdot}5\times10^{-7}$M/l.

The interpretation of the results is rendered difficult as the equilibrium surface tensions cannot be established with certainty so that the Gibbs' equation cannot be employed to determine a relation between bulk concentration and surface excess. The experiments recorded in Figures 3 and 4 may be used, however, to determine the type of system. At the end of 200 min, the surface tensions of the solution with a monolayer spread at the surface and that containing dissolved hexadecanol are equal. Assume that these surface tensions represent the same surface concentration of *all* components including hexadecanol; then, in each the surface amount of hexadecanol is one molecule per 50 Å^2 or $3{\cdot}30\times10^{-10}$ mole cm^{-2}. The first model assumes the liquid to be free from convection and of infinite depth. Provided there is no desorption of hexadecanol and the surface is not fully occupied, then the amount, Γ, diffusing to the surface at a time t is

$$\Gamma = 2c_0(Dt/\pi)^{\frac{1}{2}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where c_0 is the initial concentration of hexadecanol and D the diffusion coefficient. The diffusion coefficient at 40 °C was calculated from the Einstein-Smoluchowski

equation ($D=8\cdot0\times10^{-6}$ cm^2 sec^{-1}). Equation (**1**) gives a surface amount of $2\cdot5\times10^{-10}$ mole cm^{-2} compared to $3\cdot3\times10^{-10}$ mole cm^{-2} when the hexadecanol was spread on the surface (Fig. 4).

Applying this model to solutions of hexadecyl sulphate containing 3×10^{-4}M per litre to which have been added hexadecanol, it is more difficult to choose conditions where the equation may apply, that is, when the amount diffusing into the surface is much less than a monolayer. Five points *U*, *V*, *W*, *X*, and *Y* were chosen in Figure 3. Table 1 shows that the adsorption calculated from equation (**1**) are either vastly in excess of a monolayer or close to a monolayer. The model appears to be unreasonable.

TABLE 1

AMOUNT ADSORBED (MOLE CM$^{-2}\times10^{10}$) ACCORDING TO EQUATIONS (**1**) AND (**2**) ASSUMING $2l=0\cdot19$ CM

A complete monolayer (20 Å^2/molecule) is $8\cdot3\times10^{-10}$ mole cm^{-2}

Point: / Equation	*X*	*Y*	*U*	*V*	*W*
(**1**)	23	41	7·1	12·2	23
(**2**)	23	25	3·4	4·3	4·3

The second model is that of diffusion through an unstirred layer of thickness $2l$ adjacent to the surface and the remainder of the liquid convectively stirred. Again assume no desorption: then the amount entering the surface is

$$\Gamma=\frac{Dc_0}{2l}\left[t-\frac{2l^2}{3D}-\frac{8l^2}{\pi^2D}\sum_1^\infty\frac{(-1)^n}{n^2}\exp\left(-n^2\pi^2Dt/4l^2\right)\right]. \quad \ldots. \quad (2)$$

This equation will overestimate the amount in the surface at longer times because no account is taken of the finite capacity of the surface. From the condition above that the surface tensions are equal at 200 min for a solution with a monolayer on the surface and for another solution containing dissolved hexadecanol (Figs. 3, 4), we take $\Gamma=3\cdot3\times10^{-10}$ mole cm^{-2} and find $2l$ to be 0·19 cm. This value is approximately that found in some other systems by Sutherland (1952). It would indicate that the experimental precautions to prevent convection were unsuccessful and this is supported by experiments in which the solution below the surface is stirred by rolling an immersed glass rod back and forth. The rate of fall in surface tension was unaltered.

Applying equation (**2**) to the results for 3×10^{-4}M per litre of sulphate at points *U*, *V*, *W*, *X*, and *Y*, Table 1 shows that a reasonable fit is obtained at the shorter times.

We can now estimate the magnitude of an energy barrier which would be important at the surface if the liquid was convectively stirred. After a time greater than $2l^2/3D$ (=800 sec), the system will behave as two impedances in series, that is, there is steady diffusion through a liquid layer of thickness $2l$ and

an energy barrier at the surface. Let the concentration just below the surface be c_s, then

$$D(c_0-c_s)/2l=c_s \exp(-\Delta G/RT), \quad \ldots\ldots\ldots\ldots \quad (3)$$

where ΔG is the free activation energy for transfer from solution to surface. In the absence and presence of this energy barrier, the amounts transferred are:

$$\Gamma_a=Dc_0t/2l, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

$$\Gamma_b=D(c_0-c_s)t/2l, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

so that using (3)

$$\frac{\Gamma_a-\Gamma_b}{\Gamma_a}\times 100=\frac{100}{1+(1/D)\exp(-\Delta G/RT)}, \quad \ldots\ldots\ldots \quad (6)$$

where (6) gives the percentage decrease in adsorption at time t due to the barrier. If the results can be interpreted with an accuracy of 10 per cent., an energy barrier of 500 mV (approximately $8RT$) could just be detected. Such a potential at an air-solution interface would be unusually large and would require one ion per 120 Å^2 without any counter ions being in the surface. Phillips and Rideal (1955) found surface potentials not exceeding 200 mV for sodium docosyl sulphate on 10^{-4}M HCl and it is unlikely therefore that experimental arrangement of the type used in this work could detect the electrical energy barrier to adsorption.

The addition of salts to dilute solutions of hexadecyl sulphate apparently greatly increases the rate of attainment of equilibrium. The effect, however, can be attributed entirely to the swamping of impurity—most certainly traces of electrolytes—present in the water and the paraffin chain salt. A crude check can be made on the diffusion of alkyl sulphate in solutions containing sodium chloride. Aniansson (1951) has determined directly the amount of hexadecyl sulphate adsorbed from solutions containing 100 times as much sodium chloride as hexadecyl sulphate. Assume a relation of the form $\Gamma=kc^n$, where Γ is the surface excess, c the concentration of alkyl sulphate, and k and n are constants. The best fit gives $\Gamma=4\cdot04\times10^{-5}c^{0\cdot432}$, the error in fit being everywhere less than 10 per cent.

Sutherland (1952, eqn. (23)) has given the amount diffusing to a surface, allowing for back diffusion, in the model in which the bulk of the liquid is being stirred by convection except for a layer of thickness $2l$ at the surface.

The amount transported to the surface is

$$\frac{\Gamma}{\Gamma_f}=1-\sum_{n=1}^{\infty}\frac{\exp(-K\alpha_n^2t)}{m\alpha_n^2+0\cdot5+1/m}, \quad \ldots\ldots\ldots\ldots \quad (7)$$

where Γ_f is the surface excess at equilibrium; $K=D/l^2$; $m=\bar{M}/l$; α_n are the roots of the equation $\alpha\tan 2\alpha=1/m$.

The solution to the problem could be obtained only if there was a linear relation between Γ and c and an approximate relation $\Gamma=\bar{M}c$ was used to replace the derived relation $\Gamma=kc^{0\cdot432}$ over the concentration range of interest. Applying the Gibbs' equation to the relation $\Gamma=kc^n$, the variation in surface pressure is $\pi=RT\Gamma/n$. Hence, $\Gamma/\Gamma_f=\pi/\pi_f$ whatever n. In applying Aniansson's results a correction should be made for the variable amount of sodium chloride present at

each concentration. There are other difficulties as the purity of his alkyl sulphate, sodium chloride, and water are not known. Curve (*f*), Figure 7, shows the calculated fall in surface tension using the value of $2l$ (0·19 cm) derived earlier, D for hexadecyl sulphate as $8{\cdot}0\times10^{-6}$ cm^2 sec^{-1}, and $\overline{M}$ $(2{\cdot}57\times10^{-2})$ from Aniansson's data. The curve is a very poor approximation and choice of alternative values of $2l$ do not substantially improve the fit. Insofar as a conclusion can be drawn the rate of fall in surface tension appears to be a process governed by diffusion. As the rate is *faster* than this diffusion model, no question of an energy barrier arises.

(*b*) *Comparison of Electrolytes*

Magnesium is unexpectedly more effective in lowering the surface tension (Fig. 10) than is calcium (Fig. 9) at the highest concentration studied. The ferric salt (Fig. 11) is even more effective as the amount which can diffuse to the surface in, say, 10 min is extremely small and yet a lowering of the surface tension of 20 dyne cm^{-1} is recorded. The slow fall in surface tension with all of these systems seems to be directly related to the time for diffusion of these ions to the surface.

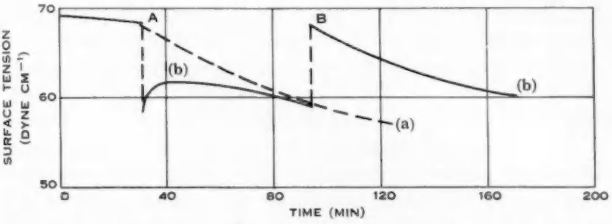

Fig. 12.—The change of surface tension with time for a solution containing $5{\cdot}0\times10^{-5}$M sodium hexadecyl sulphate/l.

Curve (*a*) gives an experimental run without change in area of surface. A second run (curve (*b*)) the surface film was compressed to 0·51 of its initial area at time *A* and expanded to its initial area at time *B*.

(*c*) *Compression and Expansion of the Adsorbed Monolayer*

Some light can be thrown on the composition of the adsorbed film by compressing and expanding it. In Figure 12, a film is compressed at a time *A* when the surface tension fell then rose steadily to a maximum and again fell to join the original curve. This implies that equilibrium cannot be reached except by a change in *composition* of the film and not merely by increasing its density. Expansion of the surface at a time *B* to the original area raised the surface tension and it appears that the composition of the film is not greatly different from that at time *A*. This would be consistent with the very small amounts of impurity electrolytes which only slowly find their way into the surface.

V. Conclusions

There appears to be very little evidence that slow falls in surface tension (or interfacial tension) are due to other than the transport of very small amounts of electrolyte impurities to a surface. The motion of liquid in a trough of some

depth can rarely be controlled and even if a thin layer of liquid in used (e.g. Sutherland 1952) it would appear that any electrical energy barriers would have to be very large to produce an appreciable change in the rate of fall of surface tension. It is probable that equilibrium surface tensions of these troublesome solutions cannot be obtained unless the concentration of the paraffin chain salt is sufficiently high (greater than 10^{-3} mole/litre) to reduce diffusion effects of inevitable impurities.

VI. Acknowledgments

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THE REACTION OF CARBON WITH HYDROGEN AT HIGH PRESSURE

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Summary

A study has been made of the reactions of a number of carbons with hydrogen at pressures up to 40 atm and in the temperature range 650–870 °C. The effect of total pressure and hydrogen partial pressure has been examined and the rate of methane formation for a given carbon can be expressed as

$$\text{rate} = kp_{H_2}.$$

Values of log k when plotted against $1/T$ give a straight line and the "apparent" energy of activation is approximately 30 kcal mole^{-1}.

The value of the constant k for a given temperature of reaction is dependent on the oxygen content of the carbon which is, in turn, dependent on the temperature of preparation of the carbon. For carbons containing no oxygen the methane rate is zero. The oxygen appears to be associated with at least two types of active centres. One type, considered to have a lactone structure, is responsible for an initial rapid evolution of methane and water and is destroyed during the first few minutes of hydrogenation. The other centre, responsible for the slow steady evolution of methane, appears to be associated with structures such as chromene or benzpyran which activate certain sites on the carbon crystallite. Neither steam nor carbon dioxide will activate the carbon for the formation of methane.

I. INTRODUCTION

Although it is known that methane is formed when hydrogen is passed through a heated bed of carbon, the existing knowledge of the physical and chemical mechanisms of this process is very incomplete.

Berl and Bemman (1932) showed that the rate of formation of methane from charcoals and activated carbons heated in hydrogen depended not only on the temperature and pressure, but on the type of carbon and suggested that the hydrogen attack took place at edge atoms. This latter suggestion has also been made by Barrer and Rideal (1935) with the observation that methane is formed only when activated adsorption is virtually completed.

At pressures above atmospheric, studies have been made of the reaction of coal chars and charcoals with mixtures of steam and hydrogen (Goring *et al.* 1951), and with hydrogen alone (Zielke and Gorin 1955). These studies show that the methane formation rate is increased by an increase in pressure but offer no satisfactory explanation for the mechanism of the reaction.

The formation of methane when carbon is reacted with steam has been ascribed to the direct reaction of the hydrogen produced with the carbon surface,

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particularly a surface activated by steam attack (Key 1948). This activating effect has also been suggested by Hebden, Edge, and Foley (1954). It has been claimed (Lewis, Gilliland, and Hipkin 1953) that the methane formed from reaction of charcoal with steam can be accounted for by the hydrogenation of basic C—H linkages in the charcoal. Recently, a further suggestion has been made (Blackwood and McGrory 1958) that the formation of methane from carbon and steam is due to the direct attack of steam on sites where hydrogen is adsorbed.

In practical gasification processes methane can be formed by a number of mechanisms. It is often difficult to assess the amount of methane formed by direct hydrogenation as distinct from that formed by other processes such as hydrogenation of unsaturated materials containing hydrogen and oxygen, or catalytic synthesis from carbon monoxide and hydrogen. The occurrence of side reactions and the presence of impurities, such as ash constituents, may also influence the formation of methane. In the present paper new experimental results are presented which show the effect of hydrogen pressure on relatively pure carbons and the effect of the chemical properties of the carbon, particularly the oxygen content, on the rate of methane formation.

II. Experimental

(*a*) *Raw Materials*

The following carbons have been used in the study of methane formation :

(i) Coconut char, extracted with hydrochloric and hydrofluoric acids, washed free of acid with distilled water, and heated in nitrogen at 950 °C.

(ii) Pure natural graphite.

(iii) Wood charcoal from *Eucalyptus marginata*, prepared at various temperatures and extracted with water.

(iv) Yallourn brown coal char, prepared at various temperatures, extracted with hydrochloric and hydrofluoric acids, and washed free of acid with distilled water.

With the exception of the graphite, which was −30, +70 mesh, B.S.S., all samples were sized −7, +14 B.S.S. Acid extractions were made as described by Blackwood and McGrory (1958), and the low ash coconut char (ash content 0·13 per cent. after treatment) was identical with the material described by these authors. The graphite was of high purity, containing 99·97 per cent. carbon. The wood charcoal was prepared by charring the raw timber, free from bark and sap wood, in a tube furnace in a continual stream of dry, oxygen-free nitrogen. Samples were prepared at temperatures of 650, 750, 850, 950, 1050, and 1150 °C, the chars being cooled in a nitrogen atmosphere. Brown coal chars were prepared in a similar manner with the exception that, after extraction with acid, and washing with distilled water, they were reheated in a stream of dry, oxygen-free nitrogen to the required temperature. Even this procedure did not eliminate all the hydrofluoric acid and for this reason analyses for carbon, nitrogen, hydrogen, and oxygen could not be made. The initial ash content

of the brown coal char, 6·5 per cent., was reduced to 2·7 per cent. by the extraction.

The chromene content of the wood charcoals was determined by the method given by Garten and Weiss (1957). Alkali adsorption was determined by heating the finely ground carbons with 0·1N sodium hydroxide solution in sealed stainless steel tubes, for 12 hr, at 130 °C. The excess alkali was filtered off and the residual carbon rinsed with distilled water; the filtrate was then titrated with 0·05N hydrochloric acid. Oxygen was determined by heating the samples in a stream of dry, oxygen-free nitrogen at a temperature above 1200 °C and collecting liberated oxides of carbon. Carbon dioxide was absorbed in "Sofnolite", and carbon monoxide liberated at the same time was oxidized to carbon dioxide, absorbed in a similar manner, and weighed separately. Analyses for carbon, hydrogen, nitrogen, and ash were made by the standard methods.

The general analysis of the wood charcoals is shown in Table 1. Gases used in the experiments were oxygen-free nitrogen, dry hydrogen, and carbon dioxide of commercial quality from cylinders and steam from distilled water.

TABLE 1

ANALYSIS OF WOOD CHARCOALS

On dry ash-free basis

Temp. of Charring (°C)	C (%)	H_2 (%)	O_2 (%)	N_2 (%)	Chromene (μ-equiv/g)
650	96·50	1·14	1·85	0·13	215
750	96·43	1·20	1·81	0·12	239
850	96·41	1·06	1·80	0·13	230
950	97·19	0·92	1·88	0·12	215
1050	97·17	0·72	1·38	0·12	162
1150	98·00	0·45	1·17	0·11	107

(*b*) *Apparatus*

Experiments were made in a flow type reactor with a fixed bed of the particular carbon. The bed temperature, gas flow rates, and pressure were controlled and measured to a high level of precision. For a full description of the apparatus and experimental technique, see Blackwood and McGrory (1958). Gas analyses were made on a modified Bone and Wheeler gas analysis apparatus and by gas chromatography on a silica gel column.

(*c*) *Procedure*

A weighed sample of dried carbon was used for each experiment, the weight of sample being such that it would occupy a length of 100 mm in the reactor tube. For the dense coconut char this was approximately 7·5 to 8·0 g and for the wood char 4·0 to 4·5 g. The reactor tube was first brought to the desired temperature and then the sample was inserted and the apparatus closed. As soon as the bed reached the correct temperature (generally within 5 min) the

apparatus was pressurized and the inlet gas flow set at the required value. Samples of the exit gas were taken at intervals as desired and the exit gas rate continually recorded. The exit gas contained hydrogen, methane, water vapour, and small amounts of ethane. The methane formation rate was determined from the flow rate and analysis of the exit gas and was expressed as moles of methane per minute per gram of carbon initially present in the bed.

(*d*) *Space Velocity*

Experiments were made, using coconut char, in which the space velocity was varied over the range 0·02 to 0·15 sec^{-1} at a temperature of 830 °C and a total pressure of 20 atm. The results are shown in Figure 1 and it can be seen that there is only a small random variation in the rate as the space velocity is increased. This means that the reaction is not controlled by mass diffusion at the surface of the carbon and that there is no appreciable retarding effect due to

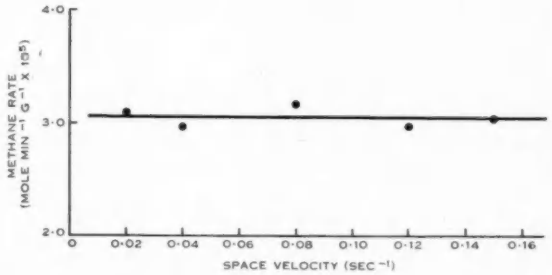

Fig. 1.—Variation of methane formation rate with change in space velocity for coconut char at 830 °C and 20 atm hydrogen.

methane formation, the methane partial pressure varying from 1·3 atm at the lowest space velocity to 0·14 atm at the highest space velocity. To facilitate analysis of the product gas samples, space velocities of 0·02 to 0·04 sec^{-1} were used for the bulk of the experimental work.

III. Experiments with Coconut Char

(*a*) *Degree of Gasification*

The experiments were designed so that the amount of carbon gasified would be small. In this way, the change in partial pressure of the hydrogen would be small and the results would be those for a differential reactor. The calculated rates were assumed to be the initial values and were calculated on the original weight of carbon in the bed. To confirm the correctness of this procedure, observations were made of the change in gasification rate with degree of gasification. For this purpose hydrogen was passed through a bed of coconut char heated to 830 °C at a pressure of 20 atm, and samples of the exit gas were taken at regular intervals and the methane rate calculated (Fig. 2). The calculated rate shows no significant change even after 10 per cent. of the carbon has been gasified. The same carbon at 650 °C showed no change even after 1 hr in the reactor when approximately 1 per cent. of the carbon had been gasified.

Since in all the experiments, particularly those at low temperature, the degree of gasification of the carbon was much less than 10 per cent., the rates measured can be taken as those for the initial carbon sample.

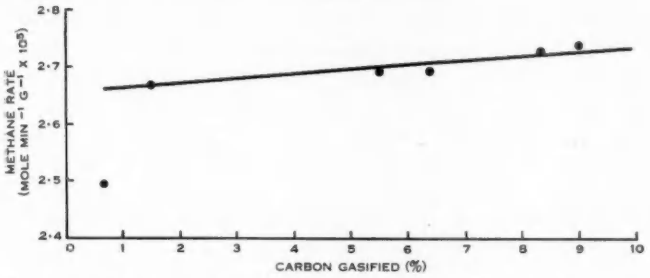

Fig. 2.—Effect of degree of gasification on methane formation rate for coconut char at 830 °C and 20 atm hydrogen.

(b) Effect of Pressure

The effects of total pressure and hydrogen partial pressure on the gasification of coconut char were examined by using mixtures of hydrogen and nitrogen in the inlet to the reactor. Thus, at 10 atm, an equimolar mixture of hydrogen and

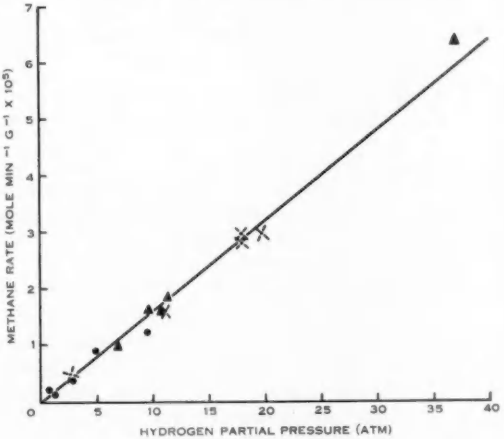

Fig. 3.—Effect of total pressure on methane formation rate for coconut char. ● 10 atm; × 20 atm; ▲ 40 atm, total pressure.

nitrogen in the inlet would give a hydrogen partial pressure of 5 atm. The run would then be repeated using a total pressure of 5 atm of hydrogen and the rates compared. Experiments of this kind were made at 830 °C in the pressure range 10 to 40 atm, and the results are shown in Figure 3. The methane formation

rate is seen to be a linear function of the hydrogen partial pressure and is unaffected by the total pressure.

Using hydrogen only in the inlet, the effect of hydrogen pressure was next examined. A series of experiments was made in the pressure range 5 to 40 atm and in the temperature range 750–870 °C (see Fig. 4). One point is included for a temperature of 650 °C. At each temperature, the rate of methane formation is a linear function of the hydrogen partial pressure.

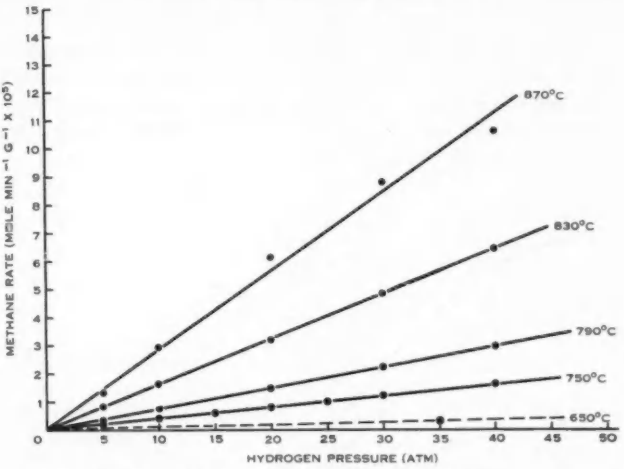

Fig. 4.—Variation of methane formation rate with hydrogen pressure at temperatures indicated.

(c) *Effect of Steam and Carbon Dioxide*

The rates of methane formation during gasification of carbon with steam (Blackwood and McGrory 1958) are considerably higher than those obtained with hydrogen alone on an equivalent hydrogen partial pressure basis. Although they showed that formation of methane by direct carbon-steam reaction almost certainly accounted for this high reaction rate, it has also been suggested (Key 1948) that steam could behave as an activating agent. Experiments were therefore made in which steam was introduced into the inlet hydrogen stream and allowed to pass through a bed of coconut char at 830 °C and 30 atm total pressure.

These experiments showed that the methane rate was not affected by the addition of small partial pressures of steam. At a total pressure of 30 atm, experiments with 3·5 and 2·6 atm of steam in the inlet hydrogen gave methane formation rates of $3{\cdot}6\times10^{-5}$ and $3{\cdot}3\times10^{-5}$ mole min^{-1} g^{-1} respectively, while the rate when no steam was present was $4{\cdot}1\times10^{-5}$ mole min^{-1} g^{-1}.

If activation was due to the removal of carbon atoms, carbon dioxide might also be expected to have a promoting effect. Experiments with carbon dioxide were made in a similar manner. It was found that when the reactant gases were introduced directly to the silica reactor tube, that is, by-passing the preheater,

so that water-gas shift was eliminated, the presence of carbon dioxide in the inlet gases did not increase the rate of methane formation, although the carbon dioxide reacted with the carbon to produce carbon monoxide. There is no relationship between the methane rate and the partial pressure of either carbon dioxide or carbon monoxide, and in the presence of these gases the rate equation is the same as for hydrogen alone.

IV. Experiments with Other Carbons

(*a*) *Effect of Charring Temperature*

It is generally considered that the type of carbon has an influence on the reaction rate of the gases with carbon and for this reason a series of wood charcoals was made as described above. A similar series of chars made from Yallourn brown coal was compared with these chars by measuring their methane formation rates under similar conditions.

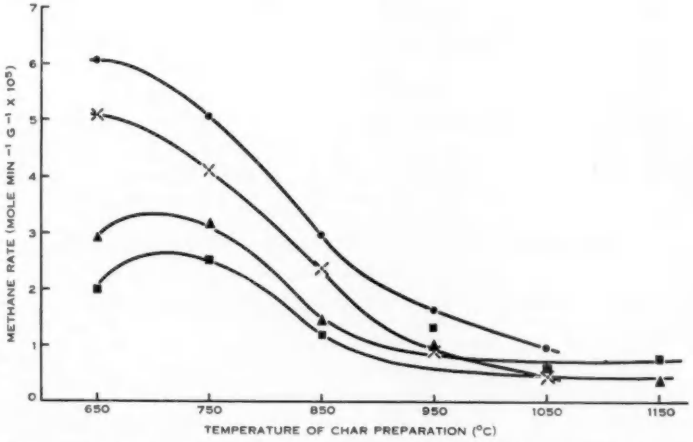

Fig. 5.—Variation of methane formation at 650 °C with temperature of char preparation at hydrogen pressures. ● Brown coal char, 50 atm ; × brown coal char, 30 atm ; ▲ wood char, 50 atm ; ■ wood char, 30 atm.

All the experiments were made at 650 °C, the lowest temperature used in the preparation of the chars to ensure that the product gas resulted only from hydrogen reaction and not from simple pyrolysis. The chars were reacted with hydrogen at total pressures of 30 and 50 atm using hydrogen only in the inlet and space velocities in the range indicated above. Samples were taken after the same time intervals in each case, 45 min, and the methane formation rates calculated. The results are shown in Figure 5. It can be seen that there is an apparent maximum in methane formation rate for wood chars prepared at about 700 °C. This maximum is shown by both series of chars and at both pressures with the exception that with the brown coal chars it occurs at a lower temperature. At higher charring temperatures the difference between the chars is less marked and they all appear to converge to a constant rate. The brown

coal chars show a higher activity than those for wood. As a comparison, coconut char, prepared at 950 °C, when reacted at 650 °C and 30 atm pressure, gave a rate of $0{\cdot}2 \times 10^{-5}$ mole min^{-1} g^{-1}.

With carbons prepared in the region 650–850 °C, the methane rate is initially very high, as shown in Figure 6. This fast reaction, accompanied by a sudden evolution of heat, lasts only for about 1 min and cannot be measured with the apparatus used due to the time taken for the gases to flow from the reaction zone to the outlet valve (about 3 min). Once this initial fast reaction is completed, the rate of methane formation becomes relatively steady, gradually decreasing

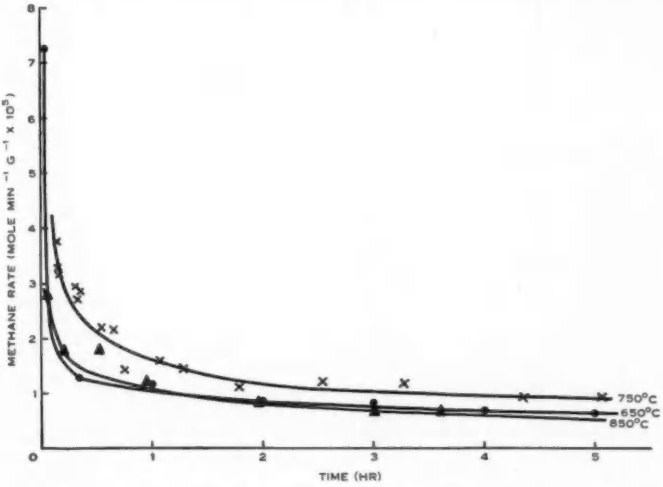

Fig. 6.—Effect of degree of gasification on chars prepared at ● 650 °C, × 750 °C, ▲ 850 °C when gasified at 650 °C and 30 atm hydrogen.

over a period of some hours. It is therefore essential that rate comparisons between chars, particularly those prepared at low temperatures, be made only over this steady period, otherwise errors due to gas sampling will affect the results.

Even when a steady rate has been reached the difference between chars made at different temperatures is apparent. Table 1 indicates that the initial oxygen content of the chars decreases with increased temperature of preparation. Experiments were made to determine whether the oxygen content of a given char decreased as the degree of gasification increased. Accordingly, the 750 °C wood char was chosen for experiment as being the most reactive and likely to show the greatest change. Four separate runs, lasting for 10 and 45 min, 2 and 5 hr respectively at 650 °C and 30 atm pressure, were made, and on completion of these runs the apparatus was quickly depressurized and cooled. The partly gasified residues were then analysed for oxygen content and chromene value.

The oxygen content decreased from 1·81 to 0·89 per cent. after the first 10 min of reaction and in 5 hr had only decreased to 0·82 per cent. The chromene value, expressed as μ-equiv. hydrochloric acid adsorbed per gram, initially 239, gave values of 250, 233, 213, and 226 for the four times respectively, and indicated no significant change. The wood chars made at other temperatures were also reacted under similar conditions for a period of 8 min and chromene values, alkali adsorption, and oxygen contents were determined on the residues. The results are summarized in Figures 7 to 10. In all cases there is a sharp decrease

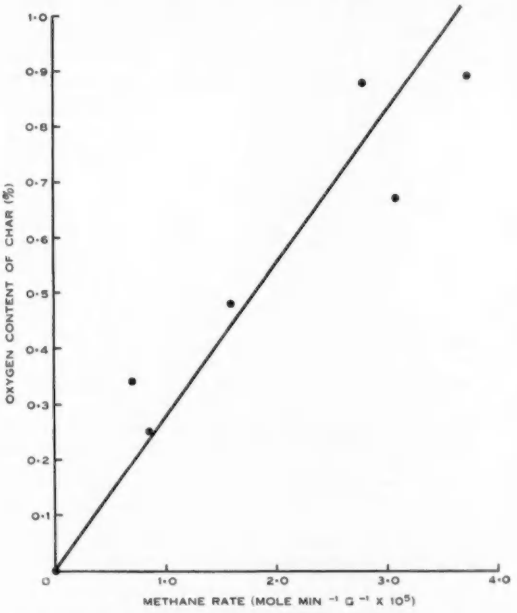

Fig. 7.—Relationship between oxygen content of wood chars and methane formation rate at 650 °C and 30 atm hydrogen.

in oxygen content corresponding to the initial fast reaction; estimation of the water liberated during this reaction shows that 2 moles of water are liberated for each mole of methane formed. Any further change is only slight, as shown by the 750 °C char. The residual oxygen content for all samples is approximately 1 per cent. less than the initial value, and at least 100 moles of methane are produced for each atom of oxygen lost during this reaction. Figure 7 shows the relationship between the methane formation rate after 8 min reaction and the oxygen content of the char at this stage. Attempts to reactivate the 650 °C residual char by heating to 250 °C in a stream of air and steam were unsuccessful, as were attempts to deactivate the original char by passing dry nitrogen over it for 12 hr.

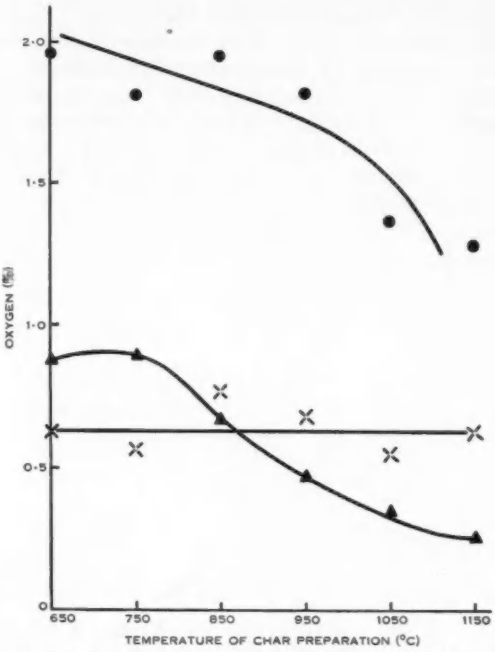

Fig. 8.—Variation of oxygen content of wood chars before and after reaction with hydrogen. ● Initial oxygen content ; × oxygen bound as —COO— and lost during initial fast reaction ; ▲ residual oxygen content after reaction for 8 min with hydrogen at 650 °C and 30 atm.

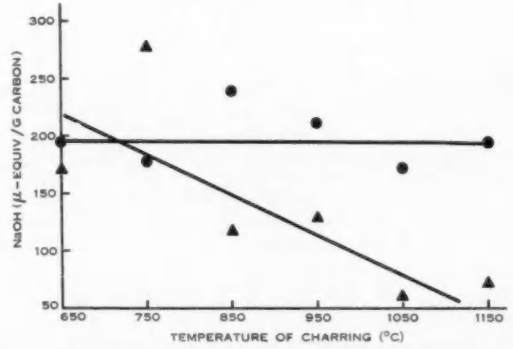

Fig. 9.—Relation between oxygen bound as —COO— and lost during initial fast reaction with hydrogen at 650 °C and 30 atm and change in alkali adsorption of wood chars during the same period. ● Oxygen lost in μ-equiv/g calculated on basis of two atoms oxygen equivalent to 1 mole NaOH ; ▲ difference in alkali adsorption before and after reaction expressed as μ-equiv. NaOH/g.

(*b*) *Hydrogen and Graphite*

The raw materials used in the above experiments are not pure carbons, although care has been taken in the selection of materials reasonably free from inorganic impurities, except silica. However, they do contain appreciable amounts of oxygen and hydrogen, and these may influence the formation of methane. As a comparison, hydrogen at 30 atm pressure was passed through a bed of graphite, consisting essentially of carbon, at 650 °C. No methane could be detected in the outlet gas.

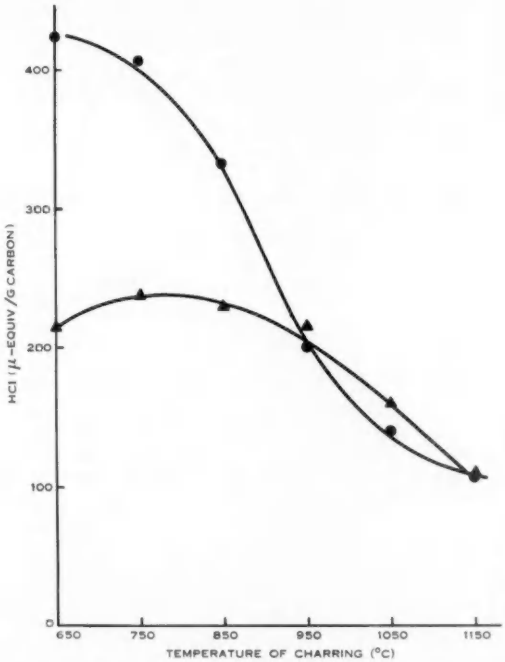

Fig. 10.—Variation of chromene value and residual oxygen content with temperature of charring for wood chars reacted with hydrogen at 650 °C and 30 atm. ● Residual oxygen content in μ-equiv/g, calculated on basis of one oxygen atom equivalent to 1 mole HCl. ▲ Chromene value, μ-equiv. HCl/g.

V. Discussion of Results

The experimental results show that for coconut char prepared at 950 °C there is a linear relationship between the rate of methane formation and the hydrogen partial pressure in the temperature range 650–870 °C. The rate of methane formation, expressed as moles of methane produced per minute per gram of carbon initially in the bed, can be expressed by the equation

$$\text{rate} = kp_{H_2}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where k is a constant and p_{H_2} the partial pressure of hydrogen in the system. The rate is unaffected by changes in space velocity and therefore is not diffusion-controlled and not retarded by the products of reaction.

A plot of log k against $1/T$, shown in Figure 11, gives a straight line between the temperature limits given above. The " apparent " energy of activation for the reaction is approximately 30 kcal mole^{-1}.

Low temperature carbons prepared in the range 650–850 °C show an initial very high methane rate which is of short duration and, with the present apparatus, is not measurable with any precision. High temperature carbons show a similar but much more rapid drop and reach a steady state in a shorter time. If the

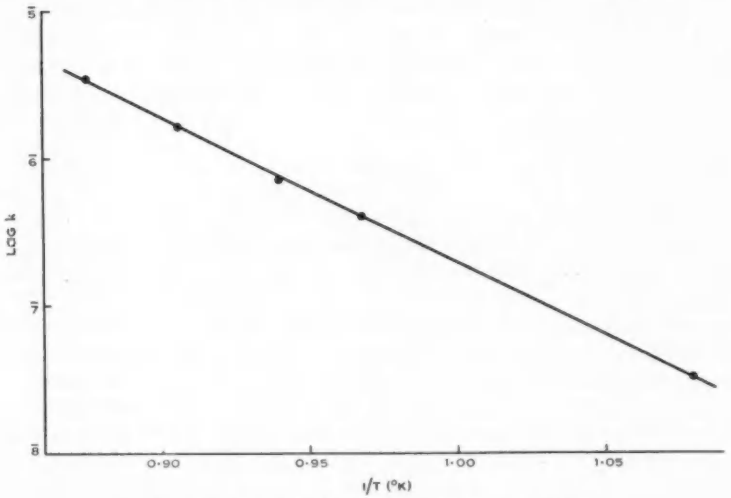

Fig. 11.—Plot of log k against $1/T$ for coconut char.

rates are measured after the fast initial reaction has ceased and a relatively steady state has been reached, the relationship shown in equation (**1**) is true for the other carbons examined.

Since the rate of reaction is a linear function of the hydrogen partial pressure over a wide range, if the mechanism is to be considered as a chemisorption process followed by reaction and slow desorption of methane, then the fraction of the surface, θ, covered by hydrogen must be small, and $1–\theta$ is almost unity. Since hydrogen is strongly adsorbed on the active centres of a carbon surface (Blackwood and McGrory 1958), this means that the number of active centres must be very small.

The hydrogenation of chars made at different temperatures shows that the higher the temperature of charring, the less reactive the char. The total oxygen contents of such chars also decrease with increasing temperature of preparation and for carbons such as pure natural graphite, which contained no oxygen, the methane formation rate is zero. The results indicate that methane formation

is in some way connected with the oxygen content of the char. Although the hydrogen content of the char also decreases with increasing temperature of preparation it is an almost linear decrease, unrelated to the sudden change in methane formation rate.

The experimental results indicate that methane may be formed by two distinct mechanisms, one responsible for the initial rapid reaction, one for the slow steady state. To explain this behaviour it is suggested that there are at least two major types of active centre.

The first type of centre is characterized by a rapid decrease in oxygen content of the chars, a decrease in alkali adsorption, and a rapid decrease in methane rate. The chromene value remains substantially unchanged.

During the determination of oxygen, where the carbon is heated to above 1200 °C in a stream of dry, oxygen-free nitrogen, the oxygen is liberated as both carbon monoxide and carbon dioxide. This would suggest that some of the oxygen is bound in such a way that two oxygen atoms are attached to one carbon atom. When the carbon is reacted with hydrogen, the oxygen is liberated as water and the ratio of methane molecules produced to oxygen atoms removed is found to be of the order 1 : 2 ; this provides additional evidence for the above observation. Reference to Figures 8 and 9 shows that the oxygen which can be liberated as carbon dioxide on heating decreases during the initial fast reaction period by an amount which is nearly the same for all chars. Although there is no statistical relationship between the oxygen lost during reaction and the difference in the amount of alkali adsorbed before and after reaction, the values of each, calculated as μ-equivalents per gram of carbon, assuming that two atoms of oxygen are equivalent to 1 mole of sodium hydroxide, are of the same order. During the determination of the alkali adsorption the high temperatures used may have caused attack on groups which would not normally be attacked at room temperature and the water rinse may have caused some hydrolysis of weakly acidic groups. For these reasons it is possible that the estimated alkali adsorptions are too low.

The active centres cannot be regenerated by treatment with steam and oxygen or by exposure to air. Garten, Weiss, and Willis (1957) have suggested that some of the acidity observed in carbons may be due to the presence of lactone groups, and it is suggested that the presence of such groups would offer a partial explanation of the behaviour of carbons to hydrogen. Such groups on hydrogenation would yield methane and water and could not readily be reformed.

The second type of centre is of a different character, since on prolonged hydrogenation the amount of oxygen in the char changes only very slightly and the ratio of methane formed to oxygen lost is of the order of 100 : 1. The methane formation rate changes only slowly on prolonged reaction and the chromene value shows no significant change.

The oxygen content of the chars and the methane formation rate are related during this stage of the reaction, as shown in Figure 7, and the oxygen content of the higher temperature chars is associated with the chromene value. If there is no oxygen present, the methane rate is zero. If the oxygen in the residual

chars, evolved as carbon monoxide on heating above 1200 °C, is calculated as μ-equivalents per gram of carbon, on the basis of one oxygen atom being equivalent to 1 mole of hydrochloric acid, and compared with the chromene value, there is fair agreement for the carbons prepared at temperatures above 850 °C. Even at lower temperatures they are of the same order. The discrepancy may be due to the presence of other groups and to the fact that during the oxygen determination the equilibrium is in favour of conversion of carbon dioxide to carbon monoxide. The bulk of the oxygen associated with this second type of centre is evolved, on heating above 1200 °C, as carbon monoxide, especially in the higher temperature carbons. Some carbon dioxide is also evolved but is much less than that associated with the first type of centre. Garten and Weiss (1957) have suggested that the chromene content is a maximum at about 800 °C, and reference to Figure 10 shows that for the wood chars this appears to be so.

It is suggested that the activity of carbon to hydrogen in the steady reaction period is largely associated with oxygen present in chromene or benzpyran groups, that these groups do not themselves react but serve as centres of activation, and that the removal of an atom of carbon to form a methane molecule results in the formation of another active centre. It may be possible that some aromatization occurs with a resultant decrease in methane formation rate or that a small proportion of the oxygen may be removed by attack with hydrogen. The presence of chromene groups has been suggested by Garten and Weiss (1957) to explain the effects of decreasing reactivity of carbon to oxygen in the presence of mineral acids, as observed by Frey (1955). Although acid extraction may have an effect on the absolute reaction rate, the results shown in Figure 5 would indicate that it does not affect the relative kinetic behaviour of extracted carbons.

The lack of activating effect found for oxygen carriers such as steam and carbon dioxide becomes clear. The active lactone group is destroyed by hydrogenation and cannot be reformed by oxidation with steam or carbon dioxide. Any attack by steam will be strongly inhibited by the hydrogen present and any contribution to the methane rate by direct carbon-steam reaction would only be small. Chromene groups are not substantially destroyed during the slow reaction, and the action of steam would not increase their activity. Reaction of steam with the carbon surface would still largely be inhibited by the high hydrogen partial pressure and the methane contribution from this source would be negligible.

It is not suggested that the two types of active centres described above are the only ones responsible for reaction, although the evidence is such that they appear to play a major role in methane formation.

VI. Acknowledgments

The author wishes to thank Mr. R. W. Urie, Mr. D. E. Weiss, Dr. C. S. Barnes, and Dr. V. A. Garten for their helpful discussions; and Mr. A. Ingeme for help in the experimental work.

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THE PURIFICATION OF ^{18}O–WATER

By I. Lauder* and I. R. Wilson*

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Summary

A reliable method for the purification of 15 to 20 mg samples of ^{18}O-water, as a preliminary step to the measurement of isotopic composition by a density technique, is described. The method is based on the use of copper oxide as the oxidizing agent but negligible isotopic dilution is involved as the oxygen of the oxide is obtained by electrolysing a small fraction of the water requiring purification. The total purification time is about 1 hr.

I. Introduction

An essential step, prior to the determination of the isotopic composition of water by a density technique, is the purification of the water. The removal of inorganic contaminants is usually not difficult but the removal of traces of volatile organic matter presents a very serious problem. The only method found in the literature for the purification of small quantities of water (20 mg) is described by Polanyi and Szabo (1934) and Herbert and Lauder (1938). The process involves circulating the water in the vapour state over a white-hot platinum filament. Experience shows, however, that the method is not entirely satisfactory. For the purification of heavy hydrogen water, on a macro-scale, methods employing oxidizing agents such as $KMnO_4$, Na_2O_2, CuO, and CrO_3 have been adopted by Linderstrøm-Lang, Jacobsen, and Johansen (1938), by Fenger-Eriksen, Krogh, and Ussing (1936), and by Keston, Rittenberg, and Schoenheimer (1937). These methods can be used for large samples of ^{18}O-water if a dilution factor is applied. These procedures, however, are not suitable for the purification of small samples of ^{18}O-water.

A new technique has been devised which has given good results even with heavily contaminated water. The method is based on the use of copper oxide as oxidizing agent but dilution due to the exchange of oxygen between the water and the oxide is eliminated. The oxygen of the oxide is obtained by electrolysing a small fraction of the sample of water requiring purification.

II. Experimental

The electrolysis cell (Fig. 1) is formed from a piece of Pyrex tube (8 mm O.D.). The thickened part is about 7 mm long and has a bore of 2 mm. Unless the cell has a suitable shape considerable difficulty is experienced during electrolysis due to bubbles blocking the capillary. As an aid to the selection of suitable cells, 20 mm^3 of 0·3M Na_2SO_4 solution are added. This should just fill the capillary. With the platinum wires (0·01 in. diameter) dipping about 1 mm

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into the solution on each side, the electrolytic resistance should be of the order 1200 Ω. The volumes of the system on the two sides of the cell are adjusted during construction so that the levels of the solution on the two sides of the cell remain constant during electrolysis.

Anhydrous sodium sulphate (1 mg) is added to the cell during construction of the system and serves as electrolyte. No exchange of oxygen occurs between the water and sodium sulphate under the experimental conditions. A current of 10–50 mA is maintained between electrodes. This fluctuates considerably and so a small coulometer containing dilute sulphuric acid is placed in series with the cell in order to determine easily the quantity of electricity which has passed through the cell. Water (2 mg) is electrolysed for each purification. Electrolysis occasionally stops due to a bubble blocking the capillary, but the bubble may easily be displaced by warming one side of the system with the hand. The cell is observed by a lens during the electrolysis. The oxygen liberated reacts subsequently with copper gauze in the silica tube (see diagram) which is heated electrically. The copper gauze is wrapped in platinum foil to avoid reaction between the copper oxide and the silica. The whole procedure may be described as follows.

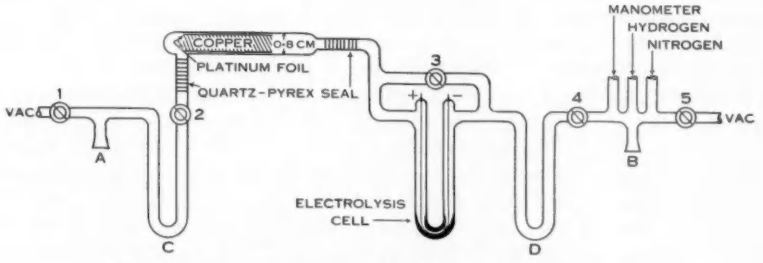

Fig. 1.—Diagrammatic representation of purification apparatus.

The vessel containing the sample to be purified is attached at *B* and the system up to tap 4 is evacuated through tap 5. The copper gauze is treated at 750 °C with hydrogen to ensure that it is free of oxide. The sample of water is pumped over the hot copper (750 °C) and is collected in U-bend *C*. During this process some organic impurities suffer thermal decomposition. The process is repeated two or three times until a Pirani gauge in the vacuum line shows no pressure rise while the vapour is passing over the copper. The latter is then cooled to room temperature and the water is condensed in the capillary of the electrolysis cell. Nitrogen is introduced to a pressure of 30 cm Hg or more. This reduces trouble due to frothing at the electrodes. With taps 4, 3, and 2 in the off position, electrolysis is started. Finally, the solution in the cell is frozen at −80 °C. The ice blocks the capillary and thus the oxygen can be held in the system while the hydrogen is pumped away. Tap 3 is then turned on to equalize the pressure on the two sides of the cell. A small heating coil is placed around the bottom of the electrolysis cell to evaporate the water to the top of the cell. If the water is simply pumped away from the cell, feathery particles of sodium

sulphate blow about the system. After allowing 15 min for the furnace to heat up to 750 °C, taps 1 and 2 are opened alternately. The oxygen is taken up by the copper and the water collects in the U-bend *C* cooled to −80 °C. The water vapour is pumped backwards and forwards over the copper oxide seven times and after the last passage it is collected in U-bend *C*. Hydrogen is introduced. The formation of water at this stage indicates sufficient copper oxide was present for the purification. All the water is transferred to a suitable vessel attached at *A*.

III. Results

The system was tested initially with the copper oxide at 400 °C, but impurities were not removed completely. At 750 °C traces of various organic compounds, methyl and ethyl alcohols, dioxane, benzhydrol, etc., were quantitatively removed as judged by the density of the sample of water purified.

At 750 °C an appreciable exchange of oxygen occurs between the quartz and the water vapour. If the quartz is initially "normal", the first sample of ^{18}O-water with an excess density of 565 p.p.m. loses 26 p.p.m. Water of normal isotopic composition passed through immediately after an ^{18}O-water sample (565 p.p.m.) turns out 26 p.p.m. heavy showing that exchange also occurs in the reverse direction. Results of this type have been obtained repeatedly. This exchange does not interfere with tracer work if three samples from any experiment are passed consecutively through the different techniques in order to eliminate memory effects.

The total purification time per sample is about 1 hr. Samples as small as 14 mg have been purified. Densities were measured with an accuracy of ± 3 p.p.m. by the modified Gilfillan-Polanyi technique described in the following paper (Lauder 1959).

A larger modification of the apparatus can be used for purification of gram-quantities of ^{18}O-water.

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THEORY AND APPLICATION OF THE GILFILLAN–POLANYI MICROPYKNOMETER TECHNIQUE

By I. LAUDER*

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Summary

An investigation of the theory and application of the Gilfillan-Polanyi micropyknometer technique (Gilfillan and Polanyi 1933), used for the estimation of the heavy forms of water, has been carried out. An improved technique was developed and the accuracy of the isotopic analysis of water by this method is now comparable with that obtained by routine mass-spectrometric analysis. The time required for one measurement is 50–60 min. The pyknometers may be calibrated by using nitrogen to change the density of the water in which the pyknometers float.

I. INTRODUCTION

The change in the concentration of deuterium or of ^{18}O used as a tracer, has frequently been determined by measuring the density of water containing the tracer. The numerous methods available have been reviewed by Kirshenbaum (1951). The micromethod, introduced by Gilfillan and Polanyi (1933), has been used by a number of workers but no discussion of the factors influencing the accuracy of the method is given in the literature. The purification of small quantities of ^{18}O-water, prior to density measurement, presented a very critical problem, but, after this had been solved (Lauder and Wilson 1959), it appeared that a detailed investigation of the Gilfillan-Polanyi method would yield useful results. As this type of pyknometer is not very well known a brief description of the construction and the method of its use is given here.

The head of the pyknometer (see Fig. 1) consists of a thin-walled glass bulb part of which has been flattened in a flame. The flattened portion allows the volume of the head to change with pressure and thus the bouyancy changes with the pressure applied. This head is joined by a solid glass rod to the body which holds about 10 mg of water. The pyknometer is filled through a small hole (0·2 mm diameter) at the extremity of the body using a vacuum technique. It is then transferred to the " equilibrium vessel " containing distilled water and totally immersed. The pressure is adjusted until the pyknometer neither rises nor falls. This pressure is recorded. Provided that the variable factors discussed in Section II are maintained constant, the difference in pressures required to make the pyknometer float when it contains one sample of water and then some other sample of water is related to the difference in densities of the two samples.

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If measurements are repeated on any one sample, using the technique described by Gilfillan and Polanyi (1933) it is found that the floating pressures show appreciable variations and drift. These disturbances can be minimized by making several floating pressure measurements on each sample of water and by bracketing measurements on each test sample with measurements on a standard sample—"normal" water. This procedure is tedious and time-consuming. A much simpler and more accurate one, resulting from the present investigation is described later.

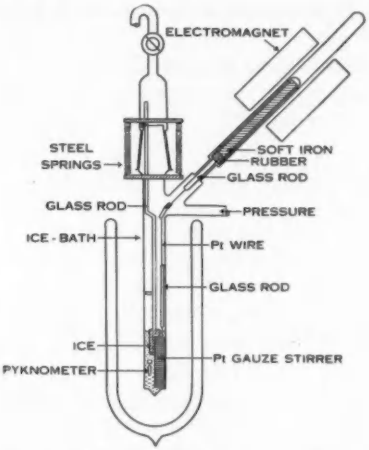

Fig. 1.—The "equilibrium vessel" showing the ice, the platinum gauze stirrer, and the submerged pyknometer.

II. Theoretical

The relationship between the density of the water in the pyknometer body and the floating pressure is obtained by applying Archimedes principle to the submerged pyknometer. The upthrust, due to the mass of the water displaced, depends on the product of two variable factors—the volume and density of the water displaced. Excluding hysteresis effects, the external volume of the pyknometer, V, is a function of the floating pressure P and the temperature T. The volume of water displaced can therefore be represented by the expression

$$V = V_0[1+\alpha(T-T_0)]+\int_{P_0}^{P}\left(\frac{\partial V}{\partial P}\right)_T \mathrm{d}P,$$

where α is the cubical coefficient of glass and V_0 is the volume at T_0 and P_0. The quantity $(\partial V/\partial P)_T$ may vary with the pressure used during measurement but this variation for a well-constructed pyknometer is usually negligible.

The density of the water displaced by the pyknometer is a function of the pressure, temperature, and amount of gas dissolved in the water. If Henry's law holds, the latter is proportional to the pressure, p, at which the water is

saturated with the gas. The density of the water may be represented by the expression,

$$d=d_0+\int_{P_0}^{P}\left(\frac{\partial d}{\partial P}\right)_{T,p}\mathrm{d}P+\int_{T_0}^{T}\left(\frac{\partial d}{\partial T}\right)_{P,p}\mathrm{d}T+\int_{p_0}^{p}\left(\frac{\partial d}{\partial p}\right)_{P,T}\mathrm{d}p.$$

The partial differential terms are constants within the range of P, T, and p existing during measurement.

The volume of water in the pyknometer body, v_0, remains virtually constant for all changes of T and P actually involved during measurement. The density of the water in the body will be affected by T and P but not by p as the water contains no dissolved gas.

By considering the conditions under which the pyknometer neither rises nor falls in the " equilibrium vessel ", it is possible to show to a first approximation, that the following equation holds :

$$d_2-d_1=(P_2-P_1)\left[d_0\left(\frac{\partial\beta}{\partial P}\right)_T+(\beta-1)\left(\frac{\partial d}{\partial P}\right)_{p,T}\right]+(p_2-p_1)\beta\left(\frac{\partial d}{\partial p}\right)_{P,T}$$
$$+(T_2-T_1)\beta\left[d_0\alpha+\left(\frac{\partial d}{\partial T}\right)_{P,p}\right], \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where P_1 is the floating pressure in normal water at a temperature T_1 and saturated with gas at a pressure p_1 when the pyknometer contains water of density d_1. For P_2 the corresponding quantities are T_2, p_2, and d_2,

$$\beta=V_0/v_0.$$

If $p_2-p_1=0$ as is the case when the pyknometer is in routine use, then $T_2-T_1=0$ and then

$$d_2-d_1=(P_2-P_1)\left[d_0\left(\frac{\partial\beta}{\partial P}\right)_T+(\beta-1)\left(\frac{\partial d}{\partial P}\right)_{p,T}\right].$$

The terms in the square brackets are constant, hence

$$d_2-d_1=(P_2-P_1)K, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where

$$K=\left[d_0\left(\frac{\partial\beta}{\partial P}\right)_T+(\beta-1)\left(\frac{\partial d}{\partial P}\right)_{p,T}\right],$$

the pyknometer constant.

The expression for the pyknometer constant is a useful one, for, if $(\partial V/\partial P)_T$ is measured before the head is sealed off during the construction of the pyknometer and V_0 and v_0 are measured, it is possible to calculate the pyknometer constant. This procedure can save time when it is desired to construct a pyknometer to work over a definite range of density. The procedure is described by Lauder (1946).

III. Sources of Error

(*a*) *Temperature*

Consider the variation in floating pressure with temperature. Suppose the pyknometer contains normal water and is floating in normal water saturated at a constant pressure. Then to a first approximation

$$d_2-d_1=(P_2-P_1)K+(T_2-T_1)\beta[d_0\alpha+(\partial d/\partial T)_{P,p}], \quad \ldots\ldots \quad (3)$$

where d_1, d_2 are the densities of normal water at the temperatures T_1 and T_2 respectively.

The temperature of the water in the equilibrium vessel rises whenever the pyknometer is introduced. This rise is estimated to be approximately 0·02 °C.

If $K=-10\times10^{-6}$ g ml^{-1} cm Hg^{-1}; $\beta=10$; $\alpha=10\times10^{-6}$ deg^{-1}, and $T_2-T_1=+0\cdot02$ °C, $(\partial d/\partial T)_{P,p}=67\times10^{-6}$ g ml^{-1} deg^{-1}, the value for water at 0 °C (Dorsey 1940), then $P_2-P_1=1\cdot4$ cm Hg.

As the floating pressure can be measured to 0·05 cm Hg, the temperature would need to be controlled to better than 0·0007 °C for a reading to this limit to have any significance.

(*b*) *Dissolved Gas*

The density of the water in which the pyknometer floats will change if the amount of dissolved gas changes. The significance of this effect can be determined from equation (**1**).

If $K=-10\times10^{-6}$ g ml^{-1} cm Hg^{-1}; $\beta=10$, $T_1=T_2$, and $p_2-p_1=1$ cm Hg, using the value for $(\partial d/\partial p)_{T,P}$ for nitrogen, namely, $-0\cdot122\times10^{-6}$ g ml^{-1} cm Hg^{-1} (Lauder 1959), $P_2-P_1=0\cdot123$ cm Hg. Thus the saturation pressure, p, must be controlled to better than 0·5 cm Hg if floating pressures, P, are determined to 0·05 cm Hg.

(*c*) *Hysteresis of Glass*

An approximate value for the hysteresis effect can be calculated from the zero point depression of a thermometer made from the type of glass of which the pyknometer is constructed. For Pyrex glass the zero point depression is 0·04–0·08 °C for a 100 °C change (International Critical Tables 1926). The time required for the glass to shrink to the volume corresponding to 0 °C depends on the type of glass. It may be from 1 up to 14 days. From the zero point depression the " effective " temperature of the glass can be calculated and hence related to the calculation of the floating pressure of a pyknometer constructed from the glass under consideration. In actual use a pyknometer is filled at room temperature and then cooled to 0 °C. The change of temperature is, say 25 °C. For this change suppose the zero point depression is 0·01 °C. From the coefficients of expansion of Pyrex glass and of mercury it is possible to calculate the " effective " temperature of the glass for this depression. The value is +0·17 °C. This means that although the actual temperature of the pyknometer is 0 °C, the total volume of the pyknometer corresponds to that for a temperature of 0·17 °C.

Suppose that two floating pressure measurements are made—the only difference between the two sets of conditions being that in the one case the pyknometer has its correct volume and in the second case, the volume corresponds to a temperature of 0·17 °C. From equation (**3**) and the data $K=-10\times10^{-6}$ g ml^{-1} cm Hg^{-1}; $\beta=10$, and $\alpha=10\times10^{-6}$ deg^{-1}, one calculates $P_2-P_1=1\cdot7$ cm Hg.

IV. Modified Technique

By far the most difficult problem is the control of temperature to the desired accuracy 0·001 °C or better. Although the lower part of the equilibrium vessel

is surrounded by an ice-bath, the heat added to the water in the equilibrium vessel, when the pyknometer is introduced or removed, is only very slowly lost. For this reason, a piece of ice frozen to the end of the supporting glass rod (see Fig. 1) is introduced and the water is stirred by the platinum gauze actuated by the electromagnet. The water is maintained saturated with nitrogen at 1 atm pressure.

The pyknometer is observed under a microscope through a clear strip in the silvering of the surrounding Dewar flask. The cone in the bottom of the equilibrium vessel serves to centre the pyknometer. The platinum gauze stirrer is lifted about half-way out of the water every 2 sec during the stirring process. The hysteresis effect is controlled by operating to a time schedule.

The volume of the water in the equilibrium vessel is about 4 c.c. When the system is first set up, dissolved air in the water is removed by evacuation. No mechanical difficulty has been experienced during 3 years of use.

The various steps in the new procedure are: (i) Fill the pyknometer by the vacuum technique; (ii) transfer the pyknometer using a glass hook to a tube containing water at 0 °C. This washes off any heavy water clinging to the outside and cools the pyknometer; (iii) transfer the pyknometer to the equilibrium vessel, the water in which is already at 0 °C and saturated at 1 atm pressure of nitrogen; (iv) introduce the ice; (v) replace the cap and displace the air by nitrogen; (vi) stir at 1 atm pressure until a total of 25 min has elapsed after cooling the pyknometer to 0 °C; (vii) stop the stirrer and determine the floating pressure within the period 25–35 min; and (viii) the pyknometer is then refilled in the period 35–50 min and the second cycle is started. On very rare occasions the floating pressure may be found to drift rapidly to higher values. The trouble is due to an air bubble sticking to the side of the pyknometer and gradually dissolving under the applied pressure. When this is experienced the pyknometer is transferred at the end of the 25–35 min period to a tube containing water at room temperature where it is left for the period 35–50 min. The standard measuring procedure is then followed. A repetition of trouble has never been observed.

No attempt is made to measure the exact floating pressure. It is usually possible to obtain within 5 min a pressure such that if it is changed by 0·05 cm Hg the direction of motion of the pyknometer will change.

V. Calibration of Pyknometers

The determination of the pyknometer constant K may be carried out by measuring the floating pressures when the pyknometer is filled with normal water and then in turn with salt solutions of known densities. The constant is calculated using equation (2). A more simple procedure is to fill the pyknometer with normal water and then to measure the floating pressures after the water in the equilibrium vessel has been saturated with gas at different pressures.

From equation (1)

$$K = -\frac{(p_2-p_1)}{(P_2-P_1)}\beta\left\{\left(\frac{\partial d}{\partial p}\right)_{P,T} + \frac{T_2-T_1}{p_2-p_1}\left[d_0\alpha + \left(\frac{\partial d}{\partial T}\right)_{P,p}\right]\right\}. \quad \ldots\ (4)$$

The temperature which exists in the equilibrium vessel during the floating pressure measurement is the temperature established while the water is being saturated at the pressure, p, in the presence of ice. Two effects enter: (i) the change in freezing point due to pressure, and (ii) the depression of the freezing point due to dissolved gas. Both effects are readily calculated and in the case of nitrogen amount to approximately $-0{\cdot}01$ °C for an increase of 1 atm pressure.

The value of the quantity in the square brackets has been determined for nitrogen using a pyknometer, L7, for which $V_0=0{\cdot}141$ ml; $v_0=0{\cdot}0087$ ml; $K=-30{\cdot}6\times10^{-6}$ g ml^{-1} cm Hg^{-1} (determined by use of a solution of sodium chloride of known density), and $\alpha=10\times10^{-6}$ deg^{-1}. It amounts to $-0{\cdot}131\pm0{\cdot}003\times10^{-6}$ g ml^{-1} cm Hg^{-1}. (A discussion of a possible error in this value is given in the following paper by Lauder (1959).) V_0 and v_0 are determined from the results of weighing the pyknometer when the body is filled with normal water and again when the water is removed. As the pyknometer floats in water at 0 °C its mean density is the same as that of water. Hence from the first weighing V_0 is obtained.

When this calibration procedure is adopted it is important to ensure that any hysteresis effect is negligible. The pyknometer should be maintained at 0 °C for 24 hr before measurements are made. It should also be noted that the procedure determines the value of K over a limited pressure range. In actual use a pyknometer may be employed over a much wider pressure range and the constant should be checked over this range. Accurately diluted heavy hydrogen water samples are more convenient for this purpose than salt solutions, because the pyknometer is more readily filled with water than with salt solution. It is not necessary to know the actual density of each standardizing solution so long as the most dilute solution requires a floating pressure within the range used during calibration by the nitrogen saturation technique.

VI. Results

A series of typical results in order of measurement obtained during 1 day is shown in Table 1. Floating pressure measurements for normal or near normal samples are made by means of a measuring microscope. Other readings are estimated from a mirror scale graduated in millimetres.

The first two measurements are results obtained without refilling the pyknometer after the previous day's work. Such measurements are always carried out to "condition" the pyknometer to the working procedure after having stood overnight or longer in distilled water at room temperature. (If the pyknometer has not been used for some time or has been cleaned by hot chromic acid cleaning mixture these first two measurements may differ from subsequent "normal" measurements by 0·2–0·4 cm Hg. The effect is due to hysteresis of glass and would be absent in quartz pyknometers.) The other normal measurements made during the day were obtained after filling the pyknometer. A change of 0·1 cm Hg in the normal pressure measurements would represent an apparent change of 5 p.p.m., so that the slight variations in the normal pressure readings are insignificant for isotopic tracer work.

The reproducibility of the measurements with water of normal isotopic composition—normal water—in the pyknometer shows that the hysteresis effect is controlled by working to a standard time schedule. Quartz pyknometers have the advantage that the hysteresis effect is absent but their use does not speed up appreciably the procedure of measurement. It is found that the time required to establish temperature equilibrium after the pyknometer is placed in the equilibrium vessel is about 15 min. For routine work, the water in the equilibrium vessel is stirred for 25 min to ensure equilibrium is established with respect to temperature and amount of gas dissolved.

TABLE 1

DATA SHOWING THE REPRODUCIBILITY OF FLOATING PRESSURES FOR NORMAL WATER IN A SERIES OF MEASUREMENTS

Results with pyknometer J3a—Pyrex glass; $V_0 = 0{\cdot}08654$ ml; $v_0 = 0{\cdot}01075$ ml; $K = -22{\cdot}54 \times 10^{-6}$ g ml^{-1} cm Hg^{-1}

Floating Pressure-Scale Readings (cm Hg)		Excess Density (p.p.m.)	Floating Pressure-Scale Readings (cm Hg)		Excess Density (p.p.m.)
Normal Water	^{18}O-Enriched Water		Normal Water	^{18}O-Enriched Water	
78·53			78·54		
78·53				47·20	1411
78·39				47·82	1385
	78·00	23		78·00	23
	78·34	8		67·97	476
	47·42	1403		67·35	504
	47·65	1392		67·80	484
	77·57	43		67·42	500
			78·50		

(*a*) *Comparison with Mass-Spectrometric Method*

The measurement of the relative isotopic composition of water by the modified technique has been compared with that by a routine mass-spectrometric method. Samples of carbon dioxide were equilibrated with four lots of the same ^{18}O-water. This water was also slightly enriched in ^{17}O. Portions of two of the samples of the carbon dioxide were analysed in a Nier-type isotope-ratio mass-spectrometer. Portions of all four were reduced by hydrogen in the presence of a nickel catalyst to give water and methane. After purification of the samples of water (Lauder and Wilson 1959), the densities were measured. Sample No. 3 was used to eliminate memory effects in the reduction and purification procedures. The excess density of this sample was not measured. Results are shown in Table 2.

As the density of pure ^{18}O-water would be approximately 20/18 times the density of ordinary water, to change from excess density in p.p.m. to apparent excess atom per cent. ^{18}O, it is only necessary to divide by $1{\cdot}1 \times 10^3$ g ml^{-1}.

(A more detailed discussion of the relationship between the mass-spectrometric results and the density results is given in the following paper dealing with the application of the present technique to the determination of the partial molal volumes of dissolved gases.) The values show that the two methods have the

TABLE 2

COMPARISON OF MASS-SPECTROMETER AND DENSITY RESULTS

Mass-Spectrometer Sample	Excess Atom ^{18}O (%)	Density Sample	Excess Density (p.p.m.) with Pyknometer J3a
No. 3	0·504	No. 1 ..	556
		No. 2 ..	551
No. 4	0·506	No. 3 ..	—
		No. 4 ..	549
Mean	0·505±0·001	Mean ..	552±3

same order of accuracy but there is not the slightest doubt that the mass-spectrometric method is superior if it is available. The density technique is restricted to measurements on the isotopic forms of water. Frequently it is a difficult matter to obtain the oxygen in a given compound in the form of water without some isotopic dilution occurring. These problems are not so acute for the mass-spectrometric method of analysis. They will be discussed in a later paper.

VII. ACKNOWLEDGMENT

The author wishes to thank Dr. I. R. Wilson for valuable discussion and criticism.

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THE PARTIAL MOLAL VOLUMES OF GASES IN WATER AT 0 °C

By I. LAUDER*

[*Manuscript received September* 8, 1958]

Summary

The partial molal volumes of nitrogen, oxygen, and carbon dioxide in water at 0 °C have been determined by a new technique based on the use of a Gilfillan-Polanyi micropyknometer as a float. The value obtained for nitrogen is 37·0 ml and that for oxygen, 31 ml. The value for carbon dioxide is found to vary from 44 to 28 ml as the pressure at which the water is saturated with the carbon dioxide varies from 0 to 6 cm Hg. The changes in the density of water at 0 °C produced by saturation with nitrogen, oxygen, and air at 1 atm pressure are —9·3, +1·8, and —4·7 p.p.m. respectively. The density of air-saturated water is considerably greater than would be expected from the sum of the effects due to nitrogen and oxygen separately.

I. INTRODUCTION

Values for the partial molal volumes of gases dissolved in water or in methanol reported or derived from data in the literature have been summarized and extended by Kritchevsky and Iliinskaya (1945). Much of the information relates to work done before the turn of the present century, and for some gases large discrepancies exist for results derived by different authors. A similar lack of agreement exists with respect to the effect of dissolved air on the density of water (Dorsey 1940).

Partial molal volumes of gases in solution are calculated from the change in volume or density of the solvent which occurs when the gas dissolves. For sparingly soluble gases this change is very small and consequently consideration of experimental error becomes very important. The purpose of the present paper is to report values for the partial molal volumes of nitrogen, oxygen, and carbon dioxide derived by a new method which is based on the use of a Gilfillan-Polanyi micropyknometer as a float. The factors influencing the accuracy of measurement of density by this type of pyknometer have been considered in detail in the previous paper (Lauder 1959), and the results presented here were obtained during this investigation.

Further work is required to investigate more fully some of the effects observed but, as the Gilfillan-Polanyi micropyknometer is no longer in routine use in this laboratory, it was thought desirable at this stage to publish the results which have been obtained to avoid their loss. Owen (1953) mentions the desirability of obtaining additional results on the partial molal volumes of carbon dioxide and oxygen in water.

The method described here is admirably suited for determinations at 0 °C, because saturation and temperature equilibrium are so readily achieved. In

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order to calculate partial molal volumes from the results, it is necessary to have available solubility data for the gas. With the dilatometer method, this information is not required as a known volume of gas is dissolved in the solvent. Much more dilute solutions can be investigated by the float method than by the dilatometer method.

II. Experimental

The gases used were obtained from commercial cylinders and were used without further purification. The purity of the oxygen was stated as 99·0–99·2 per cent. with nitrogen as the chief impurity. The purity of the nitrogen was given as 99·7–99·8 per cent. and that of carbon dioxide 99·8 per cent.

The experimental procedure was based on the technique already outlined (Lauder 1959). The pyknometer was filled with normal water, that is, water of normal isotopic composition, and then maintained at 0 °C for 2–3 days to eliminate effects due to hysteresis of glass. It was then transferred to the " equilibrium vessel " (see Lauder 1959, Fig. 1, p. 33) containing water at 0 °C. After inserting the ice and replacing the cap, the air was removed by evacuation. It was found that after continuous stirring for about 30 min, with intermittent evacuation, a floating pressure was obtained which was not changed by further stirring and evacuation. The water in the equilibrium vessel was then saturated with the gas under investigation. Experiment showed that stirring for 30 min was sufficient to saturate the water. For the first measurement the water was saturated at the maximum pressure to be used. Subsequent measurements were made after the water had been stirred for 30 min at progressively lower pressures. Again experiment showed that stirring for this time was adequate for equilibrium conditions to be set up.

For nitrogen, air, and oxygen, floating pressures were obtained by adjusting the pressure of the particular gas in use after the stirring had been stopped. For measurements with water saturated with carbon dioxide, nitrogen or oxygen gas was used to measure the floating pressure. The carbon dioxide in the system was displaced and the floating pressure obtained as rapidly as possible.

III. Results

Graphs showing the variation of floating pressure with saturation pressure for a pyknometer, L7, details of which are given in Section V of the previous paper (Lauder 1959) are shown in Figures 1 and 2. The four lines converge on the same point—the floating pressure in water free of dissolved gas.

As the floating pressure may differ considerably from the pressure at which the water is saturated, the question arises as to whether the floating pressure changes appreciably during the actual measurement, due to a change in the amount of dissolved gas. For the composition of the solution in the neighbourhood of the pyknometer to change, diffusion across the gas-liquid boundary and then through 2–3 cm of liquid must occur. The gas-liquid boundary has an area approximately 0·5 cm^2. The effect was studied experimentally. The pyknometer, free from hysteresis effects, was placed in water at 0 °C saturated at 1 atm with nitrogen and the floating pressure was applied continuously over an interval of 2 hr. The results are shown in Figure 3.

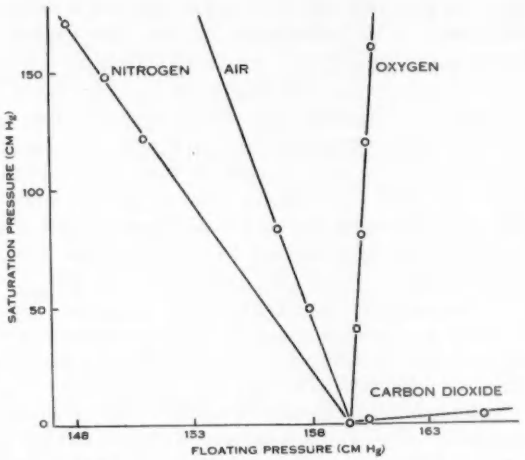

Fig. 1.—The change in floating pressure with saturation pressure for nitrogen, air, oxygen, and carbon dioxide.

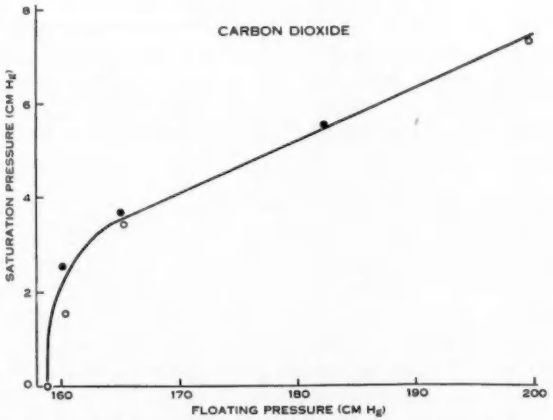

Fig. 2.—The change in floating pressure with saturation pressure for carbon dioxide.

○ Run (i); ● run (ii).

From the slope of the line the change in floating pressure in 5 min—the time required to make floating pressure readings—is 0·02 cm Hg. The effect is thus small enough to be neglected for nitrogen and also for oxygen as this gas produces very little change in the density of water, but it may be more significant with carbon dioxide.

Using equation (4) of the previous paper (Lauder 1959), the change in density of water due to saturation with gas $(\partial d/\partial p)_{T,P}$ is calculated from the curves in Figures 1 and 2.

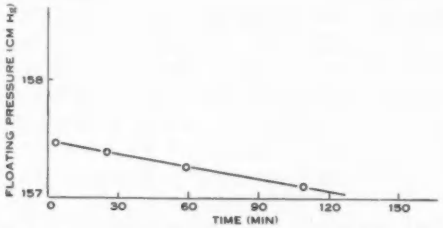

Fig. 3.—The change in floating pressure with time due to nitrogen dissolving in the water.

The values of $(\partial d/\partial p)_{T,P}$ obtained for nitrogen and oxygen are $-0{\cdot}122 \pm 0{\cdot}003 \times 10^{-6}$ g ml^{-1} cm Hg^{-1} and $+0{\cdot}025 \pm 0{\cdot}003 \times 10^{-6}$ g ml^{-1} cm Hg^{-1} respectively. For carbon dioxide over the saturation ranges 0–2 and 4–6 cm Hg, the values obtained are $+0{\cdot}8 \times 10^{-6}$ g ml^{-1} cm Hg^{-1} and $+16{\cdot}3 \times 10^{-6}$ g ml^{-1} cm Hg^{-1} respectively.

The partial molal volume φ is calculated from the expression

$$\varphi = \frac{M_2}{d_1}\left[1 - \frac{1}{c}\left(\frac{\partial d}{\partial p}\right)_{T,P}\right],$$

where M_2 is the molecular weight of the dissolved gas, d_1 is the density of the solution in g ml^{-1} at 0 °C, and c is the concentration of dissolved gas in g ml^{-1} of solution per cm Hg pressure. Data on the solubilities of the gases were obtained from Dorsey's (1940) compilation and Henry's law was assumed to apply.

From the above figures, the values for the partial molal volumes derived for nitrogen and oxygen are 37·0 and 31 ml respectively. For carbon dioxide the partial molal volume observed over the saturation range 0–2 cm Hg is 44 ml and that over the range 4–6 cm Hg, 28 ml.

IV. Discussion

The pyknometer constant for L7, $-30{\cdot}6 \times 10^{-6}$ g ml^{-1} cm Hg^{-1}, on which the accuracy of the values for the partial molal volumes depends, was determined by use of a solution of sodium chloride of known density. Although this calibration was carried out a number of years before the present investigation was undertaken, it was considered that a recalibration, to determine whether any change in the pyknometer constant had occurred, was not warranted, because

an independent check on the results was available from a comparison with mass-spectrometer results. Pyknometer J3a referred to in the previous paper (Lauder 1959) was constructed and calibrated recently using the technique described in Section V of that paper. Its constant is thus related to that of pyknometer L7. Pyknometer J3a was used in a comparison of the accuracy of measurement of the isotopic composition of water by the modified Gilfillan-Polanyi micro-pyknometer technique already described with that of the mass-spectrometric method. The experimental details and results are given in Table 2 of the previous paper (Lauder 1959, p. 39).

An approximate figure for the excess atom per cent. ^{18}O may be calculated from the density results. Allowance must be made for the ^{17}O present. The excess atom per cent. ^{17}O, as determined from the mass 45 peak of the carbon dioxide, was 0·038. No figures are available as yet for the densities of pure ^{18}O- and ^{17}O-water. As an approximation it may be assumed the densities of these forms of water are 20/18 and 19/18 respectively times the density for normal water. The ^{17}O contribution to the density then comes out to 20 p.p.m. The hydrogen used to reduce the carbon dioxide to water in the presence of nickel was electrolytic and contained only one-seventh of the normal amount of deuterium. If hydrogen of normal deuterium content had been used, the ^{18}O-water formed, would have had a density about 10 p.p.m. greater. The excess atom per cent. of ^{18}O thus comes out to 0·487. The mass-spectrometric analysis gave 0·505. The latter result is believed to be correct on an absolute basis to 1 per cent. The pyknometer constants for J3a and L7 are therefore probably too small by about 3·5 per cent. if the above assumptions are correct. (This discrepancy would not affect the use of the pyknometers for comparative isotope tracer work.) If this correction is introduced the partial molal volumes for nitrogen and oxygen become 37·4 and 31 ml respectively and for carbon dioxide 44 and 27 ml.

Kritchevsky and Iliinskaya (1945) using the dilatometer technique described by Horiuti (1931) obtained values 41, 31, and 32 ml for the partial molal volumes of nitrogen, oxygen, and carbon dioxide respectively in water at 0 °C. Other values for these gases derived by these authors from the literature show a wide scatter. The discrepancy between the value for the partial molal volume of nitrogen obtained by the float method described here, namely, 37·4 ml and that obtained by the dilatometer technique, used by Kritchevsky and Iliinskaya 41 ml, cannot be ascribed to impurities in the nitrogen. Both determinations were made with cylinder-nitrogen of similar purity without further purification. The explanation is to be sought in the experimental errors involved in the two techniques. The errors for the pyknometer technique have been discussed already (Lauder 1959).

Various sources of error involved in the use of a dilatometer for the determination of partial molal volumes of gases in solution have been discussed by Kritchevsky and Iliinskaya. These authors used a dilatometer of 100 ml capacity. The change in volume caused by saturating 100 ml of water at 0 °C with nitrogen at 1 atm pressure is +0·001 ml and so temperature control becomes very important.

Two possible sources of error are not mentioned by Kritchevsky and Iliinskaya. The volume of the dilatometer will vary slightly with pressure due to the change in the level of the mercury in the measuring capillary. Horiuti took this effect into account but Kritchevsky and Iliinskaya correct only for the compressibility of the solvent. The other source of error, in the use of a dilatometer, not discussed by Kritchevsky and Iliinskaya (or by Horiuti) is that due to the hysteresis of glass. As discussed by Lauder (1959) an estimate of the magnitude of the effect can be obtained from the zero point depression of a thermometer. If the dilatometer is made from Pyrex glass, is filled at room temperature, and then cooled to 0 °C the change in volume due to hysteresis of glass would be of the order of 20 per cent. of the change in volume produced by saturating 100 ml of water with nitrogen at 1 atm and 0 °C. Whether the effect is present in the work under discussion or not, depends on the length of time the dilatometer, filled with the solvent under investigation, remains in the thermostat bath at 0 °C before the initial reading of mercury levels is taken. The time interval is not mentioned by Kritchevsky and Iliinskaya.

The change in the value of the partial molal volume of carbon dioxide with concentration is rather surprising and requires further investigation. According to Buch (1929), Henry's law is obeyed over the range 1 to 5×10^{-5} atm and so the change in partial molal volume with pressure cannot be attributed to any irregular variation of concentration with pressure. Two experimental runs were made and the results are in reasonable agreement. Three separate effects are involved: the formation of the solution, the conversion of a fraction of the dissolved gas to carbonic acid, and the partial ionization of the latter. Kritchevsky and Iliinskaya worked at atmospheric pressure as compared with a maximum saturation pressure of 6 cm Hg for the present work and make no mention of any variation with concentration.

The value obtained for the change in density of water at 0 °C due to saturation by air at 1 atm pressure presents another surprising result. From the slope of the curve in Figure 1 and data quoted previously the value is $-4{\cdot}7$ p.p.m. atm^{-1}. Dorsey (1940) quotes a value of $-2{\cdot}5$ p.p.m. obtained by Marek (1891) but infers that this value cannot be accepted with confidence.

Using the values for the change in density of water at 0 °C for oxygen and nitrogen, $+1{\cdot}8$ and $-9{\cdot}3$ p.p.m. atm^{-1} respectively and taking the composition of air as 21 per cent. oxygen and 78 per cent. nitrogen, the calculated value for the change in density of water due to dissolved air is $-6{\cdot}9$ p.p.m. atm^{-1}. (The effects due to carbon dioxide and argon are negligible.) The solution of air in water is thus much more dense than might be expected if the effects due to nitrogen and oxygen were additive.

Possibly a more detailed investigation of the solution of unreactive gases in water would throw useful light on the nature of such solutions.

V. Acknowledgment

The author wishes to thank Dr. D. M. Alexander for useful discussion and criticism.

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OXYGEN UPTAKE AND EVOLUTION BY IRON PORPHYRIN ENZYMES

By N. Kelso King* and M. E. Winfield*

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Summary

Three detailed mechanisms are considered for the catalatic decomposition of H_2O_2. It is shown that the first of these, akin to the earlier hypotheses for catalase action, cannot satisfy the magnetic, titrimetric, and kinetic evidence. The second mechanism involves oxidation of the Fe^{III} porphyrin to the equivalent of Fe^{V}. The electron deficiency is distributed over the ligands so that even in the most oxidized complex the iron is in the Fe^{IV} or possibly even the Fe^{III} state. In the third scheme it is suggested that the reduction step (in which O_2 is liberated) takes place at a carbon atom, while the site of the oxidation is the metal atom as commonly supposed.

The liberation of O_2 from H_2O_2 can be catalysed by 6-coordinate ruthenium II complexes. In the catalytic cycle, the metal appears to be oxidized to Ru^{IV}, then reduced to Ru^{II}. Ethanol or ascorbic acid can substitute for H_2O_2 in the reduction. Evidence for H_2O_2 attack on the ligands is suggestive but not conclusive.

A brief comment is made on the bonding of oxygen to haemoglobin and myoglobin.

The accumulated evidence for the structures of catalase, peroxidase, and myoglobin complexes is utilized in a scheme for the uptake of oxygen by cytochrome oxidase.

I. Introduction

Catalase, cytochrome oxidase, and haemoglobin form a structurally related group of proteins which are concerned in oxygen metabolism. The large body of experimental studies of their chemical behaviour has not led to a formulation of the individual chemical reactions by which they fulfil their role in the living cell. Nor is a solution likely for such complex molecules without resort to analogy and hypothesis, and without a fuller understanding of the several types of bond which can form between a metal and a ligand. We have therefore attempted to set forth and discuss mechanisms which seem possible in the light of our present knowledge of coordination chemistry.

Like the enzymes which concern the metabolism of other gases (H_2, N_2, CO_2, N_2O) the proteins mentioned above are the first prerequisite of organisms which breathe, yet little or nothing is known about how they act, even though many of the subsequent reactions in the respiratory chain are known in considerable detail. There is a big incentive to learn more about the "gas enzymes",

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and of them the group which reacts with or produces O_2 is probably the most amenable to study since the non-enzymic reactions of oxygen are relatively well understood.

II. Catalase

In the 20 years since Stern (1936) determined the nature of catalase there have been many proposals for the individual steps by which it facilitates the reaction

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

There are two principal types of mechanism between which we have to choose:

$$H_2O_2 + XH_2 \rightarrow X + 2H_2O, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$H_2O_2 + X \rightarrow XH_2 + O_2; \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

and

$$H_2O_2 + XH_2 \rightarrow XH + \dot{O}H + H_2O, \quad \ldots\ldots\ldots\ldots \quad (4)$$

(which initiates a chain, ending in $\bar{O}H$),

$$H_2O_2 + XH \rightarrow XH_2 + \dot{O}OH, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

(which initiates a chain, ending in O_2 and H^+).

Most early suggestions assumed an intermediate formation of OH radicals, as in the mechanism by which ferrous ion was believed to decompose H_2O_2 (see Weiss 1952). Later it was shown experimentally that free radicals such as OH do not appear in detectable amount during the enzyme-catalysed reaction (see, for example, Chance and Fergusson 1954). Magnetic, titrimetric, and spectroscopic evidence (Section II (*b*)) has gradually accumulated to show that a mechanism of the type (**4**, **5**) is not responsible for the normal catalytic process. It cannot be excluded however that reactions such as (**4**), (**6**), and (**7**) do occur, especially when an alkyl peroxide is used as oxidant:

$$X + XH_2 \rightarrow 2XH, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

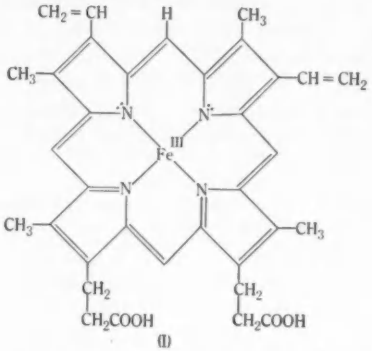
$$H_2O_2 + X \rightarrow XH + \dot{O}OH. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

In support of the 2-electron process (**2**, **3**) we have shown that thermodynamically it is more favoured than the common 1-electron scheme (**4**, **5**) which produces OH radicals (King and Winfield 1959).

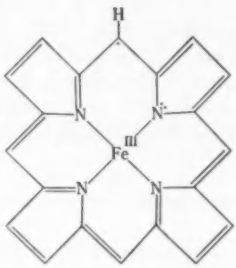
As will be seen in Section II (*a*), XH_2 proves to be an iron complex. Since H_2O_2 is believed to react with the metal atom, which is initially in the Fe^{III} state (Theorell and Agner 1943*a*, 1943*b*), we might expect that it becomes oxidized to Fe^{V}, in spite of much early evidence that the valency of the metal atom remains unchanged during the catalatic cycle (see Lemberg and Legge 1949, p. 442). By considering the structure of catalase in relation to recent work on the properties of metal porphyrins, it becomes clear how the Fe^{V} and even perhaps the Fe^{IV} state may be avoided.

(*a*) *Prosthetic Group*

The prosthetic groups of catalase are four molecules of ferric protoporphyrin (I) (Theorell 1951):



(I)



(II)

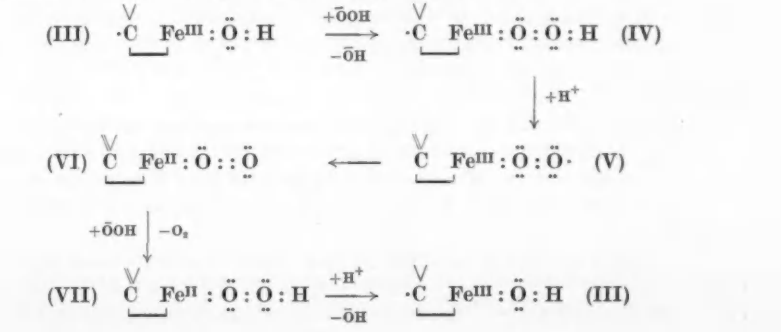
In the fifth coordination position (below the plane of the paper), the iron atom is possibly attached to carboxyl of the protein as in peroxidase, while in the sixth position (above the plane of the paper) it is attached to an $\bar{O}H$ ion (Theorell 1951).

The magnitude of the conjugated system of double bonds in I suggests that it should be possible to oxidize it to a semiquinone by removing an electron from the porphyrin. II represents one of the many canonical forms of the product.

By electron spin resonance absorption, Gibson and Ingram (1956) have shown recently that when methaemoglobin is oxidized by means of H_2O_2, an electron or a hydrogen atom is removed from the porphyrin. They have made it clear that the electron deficiency that is created is in the peripheral carbon atoms of the porphyrin rather than in the iron atom. Cahill and Taube (1951) had earlier shown that 1-electron oxidation of a phthalocyanine complex removed an electron from the resonating ring system rather than the metal atom.

(*b*) *Mechanism A*

It is now possible, without invoking the Fe^{IV} state, to write a simple mechanism for the decomposition of H_2O_2 on catalase:



in which the dot on the carbon atom represents the unpaired electron shown in complex II. The square bracket represents the indeterminate number of atoms by which Fe is linked to C in the metalloporphyrin.

Taken separately, each step in the cycle is plausible, and most steps have some experimental backing from the chemistry of myoglobin (Section II (*c*)). However, the mechanism has not proved to be acceptable. There is, for example, doubt as to whether catalase can be reduced by H_2O_2 to the Fe^{II} state. This is not necessarily a valid criticism. During the reaction cycle, which lasts 10^{-4} sec, few of the complexes shown can exist long enough to be detectable by the available

TABLE 1

MAGNETIC, TITRIMETRIC, AND SPECTROPHOTOMETRIC PROPERTIES OF CATALASE AND PEROXIDASE COMPLEXES

Complex	Unpaired Electrons per Heme	Oxidizing Equivalents* per Heme	Absorption Bands (mμ)
Cat. ..	5 (Theorell & Agner 1943*a*, 1943*b*)	0	630, 544, 505, 408 (Keilin & Hartree 1951; Herbert & Pinsent 1948)
Cat. I	3†	2‡	655, 405 (Chance & Fergusson 1954)
Cat. II	2 (Theorell & Ehrenberg 1952)		568, 536, 429 (Chance & Fergusson 1954)
Cat. III			585, 545, 416 (George 1953)
Per. ..	5 (Theorell & Agner 1943*a*, 1943*b*)	0	641, 497, 403 (Keilin & Hartree 1951)
Per. I	3 (Theorell & Ehrenberg 1952)	2 (Chance 1952)	665, 410 (Chance & Fergusson 1954)
Per. II		1 (George 1953)	555, 527, 418 (Chance & Fergusson 1954)
Per. III	2 (Theorell & Ehrenberg 1952)		583, 546, 416 (Chance & Fergusson 1954)

* Relative to free catalase or peroxidase, per heme that is oxidized.

† Since the value for Cat. I has proved too difficult to determine directly, it is assumed to be the same as that for the spectroscopically similar peroxidase complex.

‡ The mode of preparation of Cat. I and the kinetics leave little doubt that the value is the same as for Per. I.

methods (for descriptions, see Chance 1951). In a rapid cycle it is not only likely but also necessary that some of the complexes in the sequence should be unstable. As an example, complex VII must be unstable if it is to rapidly split off $\bar{O}H$.

Free catalase (Cat.) is known to contain five unpaired electrons per heme (Table 1). It is therefore not present in mechanism A, and this is contrary to the experimental evidence (Chance and Fergusson 1954). It would be necessary for Cat. to enter the cycle by a 1-electron oxidation which converted it to complex III.

A further objection is provided by the careful magnetic measurements of Theorell and Ehrenberg (1952) with Per. I, which suggests that the analogous Cat. I (the oxidized form of Cat. that is detectable spectroscopically during H_2O_2

decomposition) possibly has three unpaired electrons in the metal porphyrin that is oxidized (Table 1). An oxidation state at least as high as Fe^{IV} is thus indicated.

In making deductions from the information of Table 1, it has to be kept in mind that the kinetic evidence (mainly provided by Chance and by Theorell in a long series of papers) shows for both catalase and peroxidase that the type I complexes can be formed from the free enzyme by the action of either H_2O_2 or CH_3OOH, and that the type I complexes are the more oxidizing by two equivalents. In the catalatic mechanism, Cat. and Cat. I are the only detectable members of the cycle. Cat. I is reduced back to Cat. by the action of alcohols, or of H_2O_2 if alcohols are absent.

(*c*) *Catalatic Action of Myoglobin*

Mechanism A is little different from that suggested by Keilin and Hartree (1950) on the basis of simple spectroscopic observations of the changes induced by addition of H_2O_2 to methaemoglobin or metmyoglobin (Mb^{III}). They were able to clearly observe the appearance of an oxidation product Mb^{IV} (which at that time they believed to be $Mb^{III}-OOH$) and a little later the appearance of oxymyoglobin, $Mb^{II}-O=O$. It is of considerable interest that Keilin and Hartree were not able to detect $Mb^{II}-O=O$ until all the Mb^{III} had been oxidized to Mb^{IV} (see oxidation of ruthenium complexes, Section II (*j*)). No oxygen evolution could be detected until $Mb^{II}-O=O$ began to be visible. Further, it is relevant to the mechanism of peroxidase action (Section II (*i*)) that addition of a hydrogen-donor such as ascorbic acid rapidly reduced the Mb^{IV} to Mb^{III} while the $Mb^{II}-O=O$ remained unchanged.

On current views, Keilin and Hartree's catalatic reaction would be:

$$\underset{\underline{\quad}}{C}\ Fe^{III}-OH_2+H_2O_2 \longrightarrow \overset{\vee}{\underset{\underline{\quad}}{C}}\cdot\ Fe^{III}-OH+\dot{O}H+H_2O, \quad \ldots\ (8)$$

$$\overset{\vee}{\underset{\underline{\quad}}{C}}\cdot\ Fe^{III}-OH+\bar{O}OH \longrightarrow \overset{\vee}{\underset{\underline{\quad}}{C}}\ Fe^{III}-OOH+\bar{O}H, \quad \ldots\ldots\ (9)$$

$$\overset{\vee}{\underset{\underline{\quad}}{C}}\cdot\ Fe^{III}-OOH \longrightarrow \overset{\vee}{\underset{\underline{\quad}}{C}}\ Fe^{II}-O=O+H^+, \quad \ldots\ldots\ (10)$$

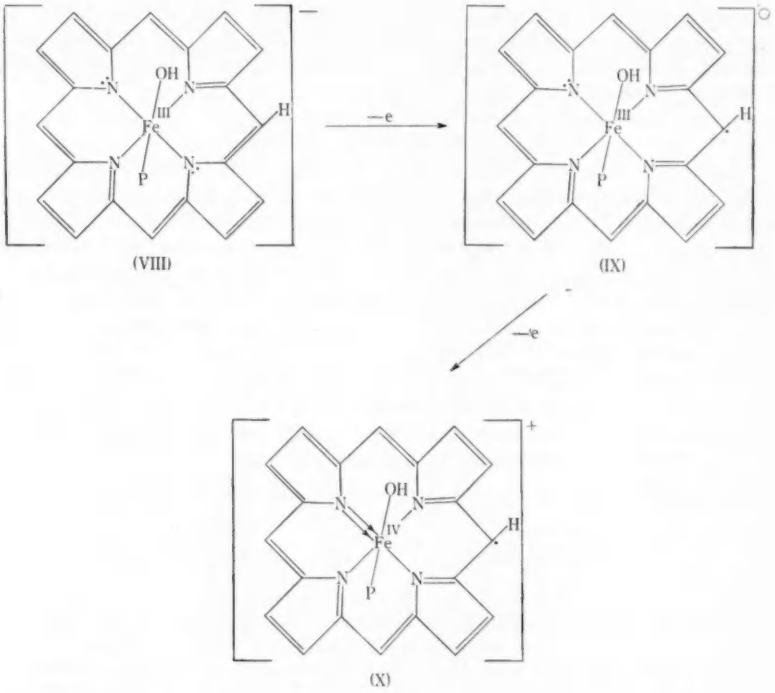
$$\overset{\vee}{\underset{\underline{\quad}}{C}}\ Fe^{II}-O=O+H_2O \longrightarrow \overset{\vee}{\underset{\underline{\quad}}{C}}\ Fe^{II}-OH_2+O_2,$$

in which the first step is that proposed by George (1952). The important features of the myoglobin-catalysed decomposition of peroxide are the valency change of the iron atom, and the strong suggestion that the oxygen is liberated from the metal rather than a carbon atom.

(*d*) *Mechanism B*

Many chemists are reluctant to accept structures which contain iron in the Fe^{IV} state. It must be remembered, however, that the formal valency assigned by convention to a complexed metal atom is not necessarily a guide to its stability

or redox potential, which are determined to a large extent by the ligands and the nature of their bonds to the metal. King and Winfield (1959) have pointed out that Co^{IV} complexes, for example, are not necessarily oxidizing agents. Also that Fe^{IV} in iron porphyrins may be stabilized by " coordinative " π bonding (both pairs of electrons contributed by the ligand). Perhaps this can be made clearer by drawing the canonical structures VIII, IX, and X.



The ligand P is a protein group. It will be seen that loss of the second electron, to give X, is not entirely borne by the iron atom but is shared by the porphyrin, whose electrons move in towards the centre of the molecule. We wish to emphasize that structure X is no more than a suggestion, and that the correct structure will be difficult to establish by experiment. The coordinative π bond could be provided by the oxygen atom (as in the ferrates mentioned below) or in part by both oxygen and nitrogen.

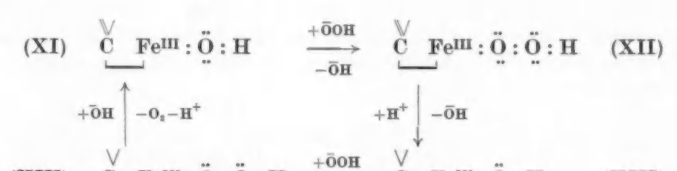
It is true in general that iron, cobalt, and nickel most resemble their higher analogues in Group VIII when they form inner orbital complexes, and resemble each other in simple salts or outer orbital complexes, as stated by Nyholm (1953). Thus, in its inner orbital complexes iron can be expected to show resemblances to ruthenium, for which the most stable oxidation states tend to be III and IV.

Over and above these considerations we have to take heed of experimental evidence, from various sources dating back to the beginning of the century, which points to the existence of high oxidation states even in simple compounds of iron. It has long been considered that the perferrites contain the ion $(FeO_3)^{2-}$, and therefore iron in the Fe^{IV} state (see, for example, Kleinberg 1950). In ferrates, such as K_2FeO_4, the metal is said to be in the Fe^{VI} state, and this is supported by magnetic measurements taken by Hrostowski and Scott (1950).

From largely kinetic evidence Bray and Gorin (1932) have deduced that the ferryl ion $(FeO)^{2+}$ occurs as an intermediate in certain oxidations of ferric ion in aqueous solution. They have gone so far as to claim that the following is a reversible and rapid reaction:

$$2Fe^{III}+H_2O \rightleftharpoons Fe^{II}+(FeO)^{2+}+2H^+.$$

This is virtually a disproportionation of Fe^{III} into Fe^{II} and Fe^{IV}. If we accept the Fe^{IV} state as reasonable in oxidized catalase, we can write for the catalatic cycle

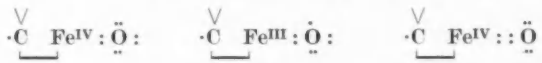


where Cat. and Cat. I are represented by XI and XIII respectively. Other complexes shown are taken to be present in undetected concentrations.

When the assumption is made that Cat. I is an inner orbital complex while Cat. is outer orbital, mechanism B satisfies the magnetochemical evidence (Table 1).

(e) *Nature of the Ferryl Ion*

Although in mechanism B we have shown the oxidized iron atom in Cat. I as Fe^{IV}, this is a matter of convenience in indicating the extent of oxidation and a result of the difficulty in representing several forms by a single structural formula after transferring the proton to another site on the molecule. Complex VIII can perhaps more accurately be represented by writing the three structures



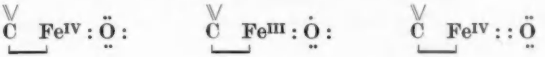
(XV)

Of these XV is close to the mean* and is of interest in that it would be a more satisfactory representation than XIII to those who feel that the absorption

* Keeping in mind that the electron distribution about the iron atom in $Fe^{IV} \leftarrow O$ can be much the same as in a ferrous complex which contains an orthodox dative π bond.

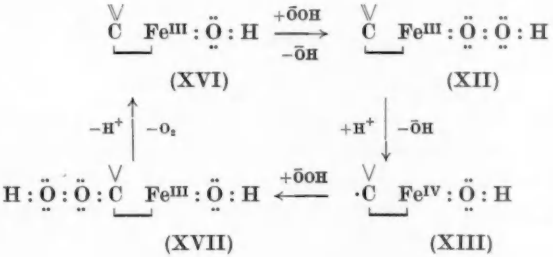
spectra indicate Cat. I to be a ferric complex. However, we are uncertain whether it can be a canonical form in resonance with the other two.

It should be noted that a mechanism involving Fe^{IV} was suggested by George (1952) prior to Ingram and Gibson's (1956) discovery of the nature of oxidized methaemoglobin. The ferryl ion structure mentioned by George as one of the several possibilities for Cat. II, and written by him simply as $(FeO)^{2+}$, presumably is indentical with the complex which we would write as the three structures



(*f*) *Mechanism C*

Chance (1951) is of the opinion that during the enzymic decomposition of H_2O_2 the peroxide becomes attached to a carbon or nitrogen atom of the porphyrin as well as to the iron atom. Structural models suggest that a peroxo bridge between iron and either carbon or nitrogen is unlikely. The following scheme is a development of mechanism B which provides for reaction at both an iron and a carbon atom, but without bridging. It retains the same interpretation of the magnetic measurements.



Here XVI and XIII are Cat. and Cat. I respectively, while XII and XVII are considered to decompose too rapidly to be detectable.

The mode of O_2 liberation shown in mechanism C finds a parallel in the reactions:

$$ROOH + Ce^{IV} \rightarrow Ce^{III} + R\dot{O}_2 + H^+$$

$$R\dot{O}_2 + Ce^{IV} \rightarrow Ce^{III} + R^+ + O_2$$

given by Tobolsky and Mesrobian (1954) as the probable course of oxidation of simple hydroperoxides by ceric ion.

(*g*) *The First Peroxide Complex of Catalase*

It has been assumed by most authors that Cat. I is a complex of peroxide ion with catalase, and that it is the anticipated enzyme–substrate complex of the Michaelis-Menten theory of enzyme kinetics (see, for example, Chance and Fergusson 1954). Chance has shown, however, that Cat. I does not necessarily

contain peroxide, even though it is the " Michaelis compound " in the sense that it " dominates the activity–substrate concentration relationship " (Chance and Higgins 1952 ; Chance and Fergusson 1954). The fact that the dissociation constant of the apparent catalase–hydrogen peroxide complex is less than 10^{-7} argues against a structure containing peroxide as a ligand (Chance *et al.* 1952).

In Table 2 are listed all the catalase complexes which we are able to conceive as products of reaction with H_2O_2, making the reasonable assumption that Fe^{I} and Fe^{V} complexes are unlikely. Whether the reactive carbon atom is in the protein or the porphyrin is immaterial to the present argument. Products in which the protein has been damaged by severe oxidation are not considered.

Of the complexes shown, the seventh (complex XIII of mechanisms B and C) appears to us to be in harmony with the published kinetic, magnetic, and titrimetric results for Cat. I and Per. I. No other complex which we have been able to write fits the available evidence, unless it be assumed that the fifth coordination position on the iron atom is unfilled (the one commonly considered to be occupied by a group of the protein). It is then necessary to consider the complexes

$$\underset{\underset{\text{C}}{\vee}}{\underline{\quad}}\ Fe^{III(5)}-OOH \qquad\qquad HOO-\underset{\underset{\text{C}}{\vee}}{\underline{\quad}}\ Fe^{III(5)}-OH$$

which would have 2 or 4 oxidizing equivalents and, if inner orbital, would have three unpaired electrons. Objections are the negligibly small dissociation constant of Cat. I mentioned above, and the difficulty of excluding water from the fifth coordination position, even if the protein is sterically prevented from attachment to the iron atom.

(*h*) *Alcohol Dehydrogenation*

Kaplan (1954) considers that in the dehydrogenation of aqueous ethanol by bromine, the likely mechanism is :

$$CH_3CH_2OH+Br_2\rightarrow CH_3\overset{+}{C}HOH+HBr_2^-,$$
$$CH_3\overset{+}{C}HOH\rightarrow CH_3CHO+H^+,$$
$$HBr_2^-\rightarrow 2Br^-+H^+.$$

The analogous mechanism

$$CH_3CH_2OH+\cdot\underset{\underset{\text{C}}{\vee}}{\underline{\quad}}\ Fe^{IV}-OH\longrightarrow CH_3\overset{+}{C}HOH+H\underset{\underset{\text{C}}{\vee}}{\underline{C}}\ Fe^{III}-OH$$

(XIII)

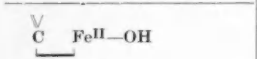
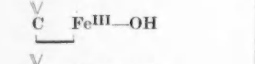
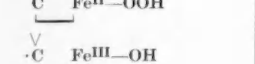
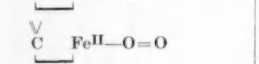
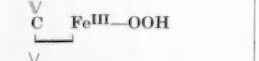
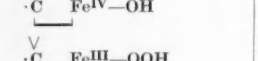
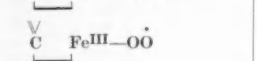
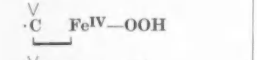
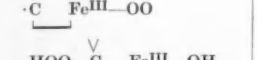
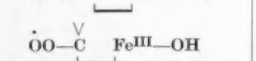
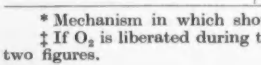
$$CH_3\overset{+}{C}HOH\longrightarrow CH_3CHO+H^+$$
$$H\underset{\underset{\text{C}}{\vee}}{\underline{C}}\ Fe^{III}-OH\longrightarrow \underset{\underset{\text{C}}{\vee}}{\underline{\quad}}\ Fe^{III}-OH+H^+$$

(XVI)

could be written for dehydrogenation of alcohol by oxidized catalase (Keilin and Hartree 1945) if mechanism C correctly describes the catalatic reaction. Direct

TABLE 2

COMPARISON OF PARAMAGNETISM AND OXIDATION STATE FOR HYPOTHETICAL AND OBSERVABLE COMPLEXES OF CATALASE AND PEROXIDASE

Hypothetical Complex	Mechanism*	Number of Unpaired Electrons		Number of Oxidizing Equivalents	Experimentally Observable Complex	Probable Experimental Values†	
		Inner Orbital	Outer Orbital			Unpaired Electrons	Oxidizing Equivalents
		0	4	—1			
	B, C		5	0	Cat., Per.	5	0
	A	0	4	1			
	A	2	6	1	Cat. III, Per. III	2 ?	1 ?
	A	0	4	1 or —1‡			
	B, C	1	5	2	Cat. II Per. II	2	1 ?
	B, C	3		2	Cat. I Per. I	3	2
	A	2	6	3 or 1			
	A	2	6	3 or 1			
	B	3		4 or 2			
		3		4 or 2			
	C	1	5	4 or 2			
		2	6	5 or 3			

* Mechanism in which shown. † For sources, see Table 1.

‡ If O_2 is liberated during titration of the complex with reducing agent, two fewer equivalents would be required than shown by the first of the two figures.

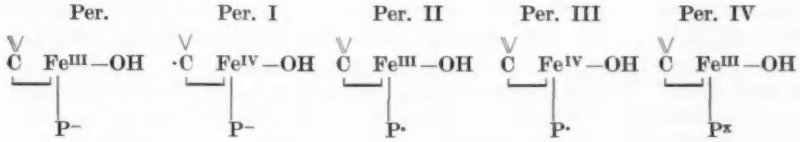
donation of electrons to the iron atom is less likely, since alcohols coordinate to metals more weakly than does water.

The assumption of two sites allows us to understand more readily why catalase is reactive towards alcohols but not phenols, while the reverse is true for peroxidase. There is little doubt that peroxidase functions by means of a two-site mechanism, one of the sites being an iron atom (see, for example, Lemberg and Legge 1949, p. 437), but the second site may be in the protein rather than the porphyrin (Section II (*i*)).

(*i*) *Peroxidase Action*

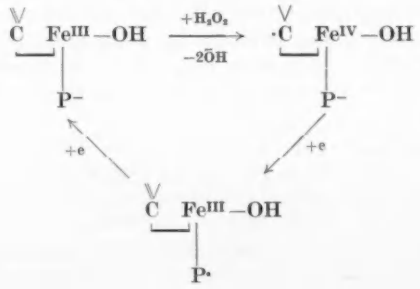
Since peroxidase contains only one molecule of iron porphyrin (Theorell 1951), there is more experimental evidence for the nature of the oxidized forms and the evidence is more readily interpreted. Similarities in the absorption spectra of catalase and peroxidase complexes indicate considerable parallelism in their reactions with H_2O_2. It is, therefore, an aid to the understanding of the catalatic reaction to examine the reactions of peroxidase, as we have already done to some extent in preceding sections.

The data of Tables 1 and 2, together with descriptions, given by Chance and by Theorell in their many papers, of how the complexes may be formed, how fast, and whether reversibly, suggest to us that the peroxidase complexes resulting from reaction with peroxides can be represented schematically thus:



where P⁻ represents the site at which the reductant attacks the protein and P· the same site after loss of one electron, while Pˣ indicates a modified protein whose state of oxidation is unknown.

Following the kinetic scheme of Chance and Higgins (1952) the oxidation of, for example, two molecules of ferrocytochrome C, with loss of one electron from each, would be represented



in which the peroxide attacks the iron atom while the cytochrome is reduced at the site P^-. The essential difference between this and the scheme for catalase is that it can provide for oxidation of a wider range of substrates and in particular those whose oxidation proceeds in single-electron steps.

(j) Reaction at a Carbon Atom

Dwyer, King, and Winfield (1959) have studied the catalytic action of certain 6-coordinate ruthenium complexes including

$$[Ru^{II}\ dipy_2\ gly](ClO_4),$$
(XIX)

$$[Ru^{II}\ acac\ dipy_2](ClO_4),$$
(XX)

and

$$[Ru^{II}\ acac\ py_4](ClO_4),$$
(XXI)

where dipy, gly, acac, and py are dipyridyl, glycinato ion, acetylacetonato ion, and pyridine respectively. When H_2O_2 is added to XX the colour can be shown to change from red (Ru^{II}) to blue (Ru^{III}) to brown (Ru^{IV}).* The brown region can then be observed to generate oxygen, with regeneration of a red complex. The valency of the metal is first increased by two units, possibly in two single-electron steps, and then decreased by two units in a two-electron step which is accompanied by O_2 evolution. Thus the catalatic mechanism resembles that described by Keilin and Hartree (1950) for myoglobin (see Section II (*c*)). With the ruthenium complexes, however, liberation of O_2 can under suitable conditions be observed when only a small fraction of the complex has been oxidized to the Ru^{IV} state, and the readily visible product of Ru^{IV} reduction by H_2O_2 is probably XX rather than its oxygenated derivative.

Complex XX can be oxidized by chlorite or ceric ion and the product reduced by H_2O_2. Alternatively, it can be oxidized by H_2O_2 and the product reduced by either ethanol or ascorbic acid (in two single-electron steps). It therefore lacks the specificity of catalase or peroxidase.

When Ru^{II} is electrolytically oxidized through blue Ru^{III} to red Ru^{IV}, the latter cannot be reduced electrolytically to Ru^{II} via the blue Ru^{III}, but instead is reduced via a green complex whose reduction is very sluggish. Chlorite ion and H_2O_2 differ from ceric ion in their oxidation effects in producing some green colour, no doubt because they permit oxidation to Ru^{III} and Ru^{IV} to be followed by partial reduction.

Due to the instability of the Ru^{IV} complex its absorption spectrum has not been reliably determined. Probably the red colour is due to absorption bands at 545 and 430 mμ, replacing the single 495 mμ band of the Ru^{II} complex. One

* In the Ru^{IV} state the complex has much the same colour as Ru^{II}; the brown colour may be ascribed to incomplete oxidation of blue Ru^{III} to red Ru^{IV}.

of the products of prolonged oxidation is a pink complex which can be restored to the original state XX only slowly if at all.

During steady-state decomposition of H_2O_2 the ruthenium complex may be largely in any one of the three oxidation states, depending upon pH and concentration of reactants. Although the complexes with which we are concerned have a redox potential which is sensitive to pH, they liberate O_2 from H_2O_2 at readily measurable rates in the pH range from 8 to 5. For complex XXI the optimum is at pH 7·4. The rate (as studied with complex XIX) is a linear function of total ruthenium concentration, and a nearly linear function of H_2O_2 concentration.

Since the rates are many orders less than for catalase, and the complexes are not rapidly destroyed by high peroxide concentrations, the kinetics are readily studied manometrically and spectrophotometrically without the many difficulties encountered in the study of catalase. By working with complexes in which all six coordination positions are occupied by strongly-held ligands, we had hoped to show that the site of H_2O_2 attack was not the metal but a carbon atom, in both the oxidation and the reduction steps. It was expected that peroxide ion would be too weak a ligand to attack in a seventh position.

The capacity of ethanol to act as reductant, although unlikely to coordinate, suggests reduction at a site on a ligand. The capacity of ceric ion to act as oxidant suggests direct oxidation of the ruthenium atom. Thus we have indications that there could be two kinds of site concerned in H_2O_2 decomposition, but the evidence is ambiguous.

Poisoning experiments are inconclusive. Low concentrations of KCN inhibit O_2 evolution in phosphate buffer at pH 7·4, while low concentrations of NH_2OH stimulate gas evolution, possibly by production of N_2. High concentrations of either KCN or NH_2OH extend the induction period, without greatly influencing the maximum rate finally obtained. Hence the ruthenium atom, in at least one of its oxidation states, is shown to be accessible to attack by cyanide ion, but the presence of cyanide does not prevent reaction with H_2O_2. Presumably KCN exerts its effect by altering the steady-state concentration of Ru^{II}, Ru^{III}, and Ru^{IV}, and the rate of attainment of the steady state. The same may be said for hydroxylamine with more certainty.

Our experiments have failed to show definitely the sites at which the ruthenium complexes are oxidized and reduced by H_2O_2, but they serve to bring to sharper focus the problems which must be solved before we can claim to understand the reactions of catalase. It is clear that in discussing the latter we have to keep in mind the possibilities:

(i) That there is more than one site for H_2O_2 attack.

(ii) That oxidations of catalase are not necessarily reversible along the same path, ligand reorganization being a prerequisite to electron acceptance (hence perhaps the origin of Cat. II and Cat. III).

(iii) That chlorite ion can yield oxidation products unlike those obtained with ceric ion and more akin to those obtained by means of H_2O_2.

(iv) That the significance of inhibition by such commonly used poisons as KCN and NH_2OH can be ambiguous.

(v) That change of valency is not necessarily accompanied by obvious colour change; in the oxidized state the catalyst is subject to changes in ligand structure, necessitating analysis by rapid spectrophotometric methods.

We wish to note here that the reaction sequence which we have suggested for catalase, and for the ruthenium complexes, is different from that which has been deduced by Nicholaev (1954) from the kinetics of H_2O_2 decomposition by 4-coordinate complexes in which the metal atom has four bonds to nitrogen atoms. He has concluded that two molecules of H_2O_2 add to the complex, followed by a recombination of oxygen atoms within the coordination sphere. Presumably this statement implies that $\bar{O}OH$ ions occupy the fifth and sixth coordination positions, and that in some way they are able to interact with liberation of O_2.

It is reasonably clear in our experiments that there is a distinct oxidation phase yielding an Ru^{IV} complex which contains no peroxide, followed by a separate reduction phase.

Wang (1955) has described a mechanism for H_2O_2 decomposition catalysed by Fe^{III}—TETA in which a peroxide ion is shown as a bidentate group. We are unwilling to accept the severe distortion of preferred bond angles which is required by two-point coordination of peroxide to a single metal atom.

III. Haemoglobin

Most oxygen carriers rely upon two metal-to-oxygen bonds and in this class there is no charge-distribution problem. Haemoglobin and the closely related myoglobin are unusual in that only one bond is formed to each O_2 molecule. No single formula for picturing the bonding has gained acceptance—the orthodox bond symbolism of coordination chemistry is inadequate and moreover it is difficult to deduce the probable charge distribution in oxyhaemoglobin.

Pauling (1949) has made several proposals for the mode of bonding. Of these, the best known is:

$$Fe^{II}=O-O.$$

The carbonyl complex is represented by $Fe^{II}=C=O$. In both, the bond between metal and ligand is made up of two electrons contributed by the ligand and two d_ε electrons of the metal (see, for example, Williams 1956). Elsewhere we have discussed the nomenclature and symbolism used to describe metal carbonyls, and have suggested that the mode of attachment of carbon monoxide is better written $M-C\equiv O$, leaving it to the reader to mentally picture the π bonding (King and Winfield 1958, unpublished data). On this convention carbonylhaemoglobin* would be written $Fe^{II}-C\equiv O$ and oxyhaemoglobin as $Fe^{II}-O=O$. In the latter, the positive charge that would seem to be imposed on the oxygen

* When it is necessary to show more detail they can be written:

$$Fe^{II}\overset{\rightarrow}{\leftarrow}C\overset{\leftarrow}{=}O \qquad \text{and} \qquad Fe^{II}\overset{\rightarrow}{\leftarrow}O\overset{\rightarrow}{-}O$$

atom adjacent to the metal is partly dissipated by donation of d_z electrons by the metal. The formula is thus not intended to represent a canonical form, but to be a convenient representation of a mixture of the structures

$$Fe^{II} :: \ddot{O} : \ddot{O} : \qquad \text{and} \qquad Fe^{II} : \ddot{O} :: \ddot{O}$$

with possibly some contribution from $>N :: Fe^{IV} : \ddot{O} : \ddot{O} :$ in which the bond from N to Fe is a " coordinative " π bond, that is, all four electrons have been donated by the nitrogen atom (King and Winfield 1958).

The oxidation reactions of haemoglobin in the presence of O_2 and hydrogen-donors, which have been described by several authors (for an able discussion, see George 1952), could be described in terms of the complexes

$$\overset{\vee}{\underset{\_\_}{C}}\ Fe^{II}-O=O \qquad \overset{\vee}{\underset{\_\_}{C}}\ Fe^{III}-O-\dot{O} \qquad \cdot\overset{\vee}{\underset{\_\_}{C}}\ Fe^{III}-O-O^-$$

(XXI) (XXII) (XXIII)

In XXI the electronegativity of oxygen is matched by that of the iron complex with which it is combined (see George 1952), so that negligible transformation of XXI to XXIII occurs. However, in the presence of a reducing agent which can attack the carbon atom we may expect

$$XXI \rightleftharpoons XXII,\ XXIII \xrightarrow{+e} \overset{\vee}{\underset{\_\_}{C}}\ Fe^{III}-O-\bar{O}$$

$$\downarrow +H_2O$$

$$\overset{\vee}{\underset{\_\_}{C}}\ Fe^{III}-OH+\bar{O}OH$$

followed by oxidation and reductions of the kind already described in the sections on catalase and myoglobin.

IV. Cytochrome Oxidase

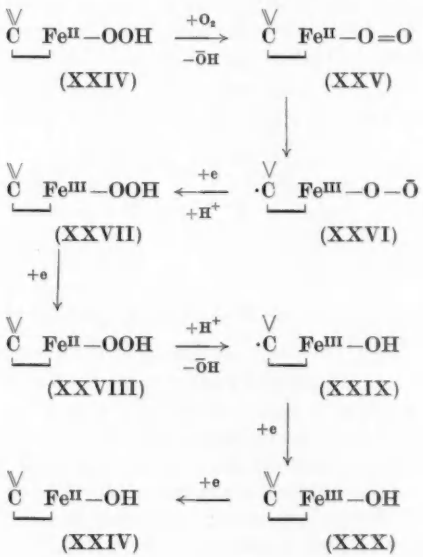
The respiratory enzyme in many organisms, both animal and plant, is a haemoprotein which is classified spectroscopically as cytochrome a_3. Our meagre knowledge of the enzyme in spite of its very great interest to biochemistry is attributable to the unusually great difficulty of purification. The prosthetic group appears to be different from iron protoporphyrin in having an aldehyde group, possibly in place of the 3-methyl (Rawlinson *et al.* 1949).

Usually cytochrome a_3 is closely associated with the auxiliary enzyme cytochrome a. In *A. pasteurianum* its place is taken by cytochrome a_1 unaccompanied by cytochrome a (Chance 1953). It is therefore reasonable to assume that the roles of combining with O_2 and of directly supplying to it the electrons needed for its reduction are solely filled by the terminal enzyme conveniently called cytochrome oxidase. Also that associated cytochromes are concerned only in the transfer of electrons from ferrocytochrome c to the oxidase, and in

generation of high-energy phosphate esters. Hypotheses for oxidase action which directly involve cytochrome a, for example that of Ball and Cooper (1953), are thus no longer favoured. In the mechanism which we wish to suggest, we indicate the electrons supplied by cytochrome c (assumed to be chemically bonded via one or more proteins to the iron porphyrin of the oxidase) but do not show the transfer system.

(a) Mechanism

Cytochrome oxidase is taken to be like peroxidase in having a protein group which acts as the site of electron input by the hydrogen donor (e.g. cytochrome a), and which can lose electrons to a neighbouring iron porphyrin attached to the same protein. But the iron atom is considered to be complexed in such a way that the Fe^{II} state is favoured rather than the Fe^{III}, showing a resemblance here to myoglobin. In its property of breaking the O—O bond with liberation of $\bar{O}H$ the enzyme resembles catalase. It is assumed, however, that the iron porphyrin is connected by a semiconductor to a continuous source of electrons, and this markedly alters the character of the reaction cycle:



Of the complexes shown, XXVI, XXVII, XXVIII, and XXIX are unlikely to build up to detectable concentrations.

The principal evidence for the iron atom as the site of oxygen attack is to be found in discussions by Warburg (1926), Ball and Cooper (1953), and Eichhorn (1956) of enzyme inhibition by carbon monoxide and sulphide. On the contrary, Williams (1956) favours attack at the periphery of the porphyrin.

V. Acknowledgments

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VI. Addendum

Since the preparation of the present paper Gibson, Ingram, and Nicholls (1958) have modified the earlier conclusions of Gibson and Ingram (1956) on metmyoglobin oxidation. They now believe that two distinct complexes result from the reaction with H_2O_2. One, the " metmyoglobin peroxide compound ", we interpret as an Fe^{IV} complex containing no peroxide. The other, the so-called " free radical ", they suggest contains an odd electron in the protein rather than in the porphyrin. If these views are correct, it is necessary to write in place of reactions (**8–10**)

$$\underset{\underline{\quad}}{\overset{\vee}{C}}\ Fe^{III}-OH_2+H_2O_2\longrightarrow\underset{\underline{\quad}}{\overset{\vee}{C}}\ Fe^{IV}{=}O+H_3\overset{+}{O}+\dot{O}H,$$

and

$$\underset{\underline{\quad}}{\overset{\vee}{C}}\ Fe^{IV}{=}O+H_2O_2\longrightarrow\underset{\underline{\quad}}{\overset{\vee}{C}}\ Fe^{III}-O{=}O+H_2O.$$

In the normal oxidation of catalase by H_2O_2 we regard the two electrons as removed simultaneously from Cat., although in complexes VIII, IX, and X a consecutive loss is shown for convenience in explanation. Whether an electron is removed first from the metal or from the porphyrin is of little significance except in the oxidation of Cat. or Per. by free radicals derived from H_2O_2.

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PAPER IONOPHORESIS OF CARBOHYDRATES

I. PROCEDURES AND RESULTS FOR FOUR ELECTROLYTES

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Summary

Details are given of procedures for effecting paper ionophoresis of polyhydroxy compounds in the electrolytes borax, sodium arsenite, basic lead acetate, and sodium hydroxide, and for detecting the compounds after ionophoresis. Rates of migration are reported for 96 compounds, including all pentose and hexose sugars, the common disaccharides, all sugar alcohols up to the heptitols, the cyclitols, a number of glycols, and several glycosides and other derivatives of carbohydrates. Some new or improved reagents have been developed for locating carbohydrates on paper strips under various conditions. Sodium arsenite and basic lead acetate are the most effective electrolytes for separating reducing sugars, basic lead acetate is the best for separating sugar alcohols, and borax is the best for simple glycols. Some success has been achieved in correlating the configurations of stereoisomers with their mobilities in paper ionophoresis.

I. INTRODUCTION

Only borate buffers have been used extensively as complex-forming electrolytes for separation or identification of carbohydrates by paper ionophoresis (for a review, see Foster 1957*c*), although several other electrolytes have proved to be useful (Barker *et al.* 1956 ; Frahn and Mills 1956*a*, 1956*b* ; Theander 1957). Rates of migration have now been determined for common reducing sugars, glycosides, sugar alcohols, cyclitols, and glycols in the electrolytes sodium arsenite, basic lead acetate, and sodium hydroxide, and compared with the rates of migration in borate buffer. The principal aim has been to develop efficient separations of carbohydrates and reliable methods for the detection of the carbohydrates after ionophoresis. It has also been possible to correlate configuration with relative rate of migration for several groups of isomeric carbohydrates.

II. PROCEDURE AND RESULTS

(*a*) *Materials*

The compounds for which data are given were, with few exceptions, pure commercial or research preparations, and unless specified they have been applied as 0·1M aqueous solutions. The chief exceptions are D-tagatose, D-psicose, and the ketopentoses, which were identified in mixtures obtained by epimerization of reducing sugars in hot pyridine, and may not be 0·1M. D-Tagatose was obtained from D-galactose and from D-talose, and D-psicose from D-altrose, D-allose, and D-fructose. The identification of the ketopentoses is less certain, as both *threo*pentulose and *erythro*pentulose seemed to be formed by the

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pyridine-induced epimerization of xylose, ribose, or arabinose. The configurations assigned to the pentuloses in Table 2 are based on the apparent relative abundance of the ketoses formed from the different aldopentoses.

The glycols listed as *erythro-* and *threo*pentane-2,4-diol were obtained as a mixture on reducing pentane-2,4-dione with sodium in alcohol (Bauer 1912). A nearly complete separation of the mixture was achieved by selective acid-catalysed reaction with *cyclo*hexanone (Mills, unpublished data), the isomer that readily affords a *cyclo*hexylidene acetal being tentatively assigned the *erythro* configuration. 2-Methylpentane-1,3-diol was a commercial product (L. Light & Co. Ltd.), apparently a mixture of isomers.

Two samples of poly(vinyl alcohol) were used, a solid (British Drug Houses Ltd.) and an aqueous solution (" Pevafix ", May & Baker Ltd.), which were applied as approx. 0·5 per cent. solutions without an attempt at purification.

(*b*) *Electrolytes*

(i) *Borax.*—It is convenient to work at the natural pH of borax solution. The solution recommended (Frahn and Mills 1956*a*) has pH 9·2 and contains 0·20 g-atom boron per l. (19·1 g $Na_2B_4O_7.10H_2O$ per l.), and will be referred to as " 0·05M borax ". No special precautions to exclude carbon dioxide seem to be needed. Platinum electrodes may be inserted directly into the electrolyte, but the anolyte and catholyte should be recombined and mixed daily.

(ii) *Sodium Arsenite.*—The electrolyte contains 0·20M arsenious acid adjusted to pH 9·6 with sodium hydroxide (19·8 g As_2O_3 in 1 l. 0·13N aqueous sodium hydroxide; Frahn and Mills 1956*a*). Some protection from carbon dioxide is desirable, and connection to the electrodes should preferably be through salt bridges. Platinum electrodes may be inserted directly into the electrolyte, but the platinum becomes brittle, some arsine may be evolved, and a brown colour develops in the catholyte, which is, however, discharged on mixing it with the anolyte. Fresh electrolyte should be prepared at frequent intervals (say after 40 hr of actual electrolysis), as its efficiency gradually falls, even though analysis for arsenite shows no obvious change in composition and pH 9·6 is maintained.

(iii) *Basic Lead Acetate.*—The material used (" lead acetate, basic, crystals "; Hopkin & Williams Ltd.) appeared to be essentially $Pb(OAc)_2.2Pb(OH)_2$, and a solution containing about 0·2 g-atom lead per l. was made by shaking 58 g of the salt with 1 l. water and filtering. The solution has pH 6·8. It should be protected from carbon dioxide. Platinum electrodes may be inserted directly into the electrolyte, and the slow electrodeposition has no detectable effect on its efficiency.

Basic lead acetate differs from the other electrolytes in affording cationic complexes with polyhydroxy compounds. It causes fairly rapid degradation of some reducing sugars, especially D-galactose (cf. Svoboda 1896) and D-psicose, even though its pH is near that of maximum stability for most sugars; therefore it is not suitable for quantitative separations.

(iv) *Sodium Hydroxide.*—Aqueous 0·1N sodium hydroxide is sufficient to effect reasonably rapid migration of reducing sugars, without the passage of

unduly high currents or excessive degradation of the sugars or the paper support. It should be protected from carbon dioxide and renewed at frequent intervals, and good cooling is essential.

(c) *Apparatus and Procedure for Runs*

The apparatus was modelled closely on the enclosed-strip apparatus described by Foster (1952). When air has to be excluded, the lids are sealed with surgical adhesive tape. The levels are adjusted through a removable siphon, filled by suction through a three-way T-stopcock. The glass plates (46 by 15 by 0·65 cm) enclosing the paper extend about 1·5 cm at each end beyond the chromium-plated brass cooling plate. The paper strips are 61 by 13·3 cm, with about 2 cm at either end in the electrolyte and 46 cm enclosed by the glass and cooled.

To exclude air during application of the test substances to the previously wetted paper, a thin sheet of poly(vinyl chloride), also 46 by 15 cm, is inserted between the paper and the upper glass plate. This sheet has 10 holes each 0·4 cm in diameter drilled in a line across the centre, starting 2·1 cm from the edge of the sheet and spaced 1·2 cm apart. A second line of five similar holes is drilled parallel to the first line and 1 cm from it, these holes being 2·4 cm apart and starting 2·7 cm from the edge of the sheet. The marker for correction for electro-osmosis may be applied, together with the standard of rate, through each hole in the line of five, and substances under examination through the holes in the line of ten. The markers and standards of rate are applied " behind " the test substances, therefore the line of five holes is placed nearer to the anode in basic lead acetate, and nearer to the cathode in the other electrolytes. After the run, each test substance is adjacent to a lane containing one marker and one standard of rate with which it may be compared (after correction for the initial 1 cm displacement of the markers), thereby largely compensating for possible " edge effects ". In very precise work it is advisable to use only the centre lanes on the paper strip.

The paper strip is always completely wetted with electrolyte before the test substances are applied. This is essential for success with sodium hydroxide and basic lead acetate ; if the test substances are applied first and the paper is then wetted with electrolyte from either end (Foster 1952) the line of junction of the two wetted areas is very deficient in electrolyte, presumably through adsorption on the paper. The use of prewetted paper permits the more efficient use of markers as described above, and also an adequate equilibration, by capillarity, of the paper and the liquid in the electrode compartments before each run. Rapid and complete mixing of test solution and electrolyte occurs on the paper even when the test solution contains hydrophobic impurities, or is supersaturated. For qualitative work, test solutions are conveniently applied with a small platinum loop, which is a well-tried device in microchemistry for transferring uniform small volumes of solutions. The loops used deposit a 2–3 mm spot on dry filter paper, and tests with coloured solutions showed that on wet paper the liquid transferred from the loops rapidly spreads to 3–4 mm diameter and diffuses only slowly thereafter.

Whatman No. 4 paper is used throughout, without previous treatment. Occasionally, a 1–2 cm strip at the edge of a sheet may contain a reducing impurity that reacts with the oxidizing reagents described below. The starting line is marked with a graphite pencil, and, contrary to previous reports (e.g. Crestfield and Allen 1955), no ill effects have been observed from the presence of this line or other pencilled notations on the paper. The paper strip is drawn through the electrolyte in a dish, drained briefly, and lightly blotted between sheets of blotting paper (" Devon Valley 431 Mill ", from Australian Paper Manufacturers Ltd.). Reproducible blotting is achieved by using a 20 cm length of rubber wringer-roller from a clothes-washing machine, fitted with a yoke and handle (total weight 1·7 kg), which is drawn across the blotter without additional pressure. The blotted paper is quickly placed on the lower glass plate and centred over a line drawn across the centre of the cooling plate, the perforated sheet is centred over the starting line and covered with the upper glass plate, and the assembly is clamped at the edges with strong spring-backed paper clips. For capillary rise of electrolyte from the tanks 30 min is allowed. The upper plate is removed, the markers and test solutions are applied through the holes, the upper plate is replaced and clamped, and the potential is applied at once. With these arrangements, when the temperature in the room is about 20–25 °C and of the cooling water about 18–20 °C, a potential difference of 1300–1400 V gives currents of less than 35 mA, except for 0·1N sodium hydroxide, for which the current reaches 50 mA. The mean potential gradient is therefore 23–25 V/cm.

After ionophoresis papers are normally dried in an oven at 100–105 °C until crisp. It was found that papers should not hang in a U-shape : the centre dries more slowly, and electrolyte from here may move out to the drier parts. Horizontal support during drying (Williams, Pickels, and Durrum 1955) is better, therefore after runs the ends of the strips projecting beyond the glass plates are torn off, and the remainder is weighted at the ends and draped over glass rails 30 cm apart, so that the part containing the spots is horizontal while being dried.

After runs with relatively volatile compounds, the paper is only partially dried in the oven, then at room temperature. The same procedure is necessary after runs with reducing sugars, even in borax, if it is desired to prevent any transformations in the hot, concentrated electrolyte during the final stages of drying. Papers wet with sodium arsenite may evolve some arsenious oxide on drying at 100 °C.

(*d*) *Differential Movements of Electrolyte on the Paper*

Electro-osmosis moves each of the four electrolytes through the paper in a direction opposite to the migration of the carbohydrate complexes. In addition, transfer of electrolyte by capillarity occurs between the paper and the tanks, because it is impossible to blot the paper accurately to the equilibrium liquid content. Slight over-blotting is preferable. When the paper is first clamped in place, a fairly rapid inflow occurs at the ends, detectable by spotting substances at different points along the paper and examining their distribution after a short time. The inflow is slow after 30 min, but may continue for up to 2 hr. Starting the runs in the centre of the paper allows most of this differential movement

to subside before the migrating complexes reach the regions of more rapid inflow. Clamping the assembly at the edges only may distort the flow pattern, both electro-osmotic and capillary, at the edges of the paper strip, but the distribution of markers across the paper largely compensates for this.

(*e*) *Detection of Carbohydrates after Ionophoresis*

Reagents used in paper chromatography of carbohydrates (Kowkabany 1954) may not be equally suitable for detecting carbohydrates after paper ionophoresis. In addition to reacting chemically with the detecting agent, the electrolyte may alter the structure of the paper and so prevent selective reaction of soluble carbohydrates in the presence of the cellulose. Some new reagents have been developed to meet the requirements of the four chemically diverse electrolytes, and some established reagents have been modified. In general, the reagents used will detect with certainty a compound initially applied as a small spot of 0·1M solution and run for 90 min ; the sensitivity is much greater for reducing sugars and sugar alcohols. A few glycols and glycosides should be applied at greater concentrations to facilitate detection. Sodium arsenite generally causes more difficulties than the other electrolytes.

(i) *Spraying of Papers.*—The atomizer, operated by compressed air, delivers about 20 ml/min as a fine spray. Normally, both sides of the paper are sprayed lightly, as this gives more uniform coverage. Displacement of spots during spraying is reduced if the borders of the area to be covered (extending well beyond the spots) are sprayed first, and the centre then quickly filled in. Any unwetted ends are torn off before the paper is heated. This is important when aqueous solutions are being sprayed, to prevent displacement of spots towards the dry areas.

(ii) *Application of Aqueous Reagents.*—Some aqueous solutions have to be applied as a spray, but a transfer technique gives superior results with certain reagents. A piece of blotting paper (" Devon Valley 431 Mill ") slightly larger than the paper strip to be developed is dipped into the reagent solution, drained briefly, and blotted lightly between sheets of Whatman No. 4 paper. The dry paper strip is placed on a sheet of glass and covered with the prepared blotting paper, and the two are pressed into intimate contact by the wringer-roller squeegee (Section II (*c*)). The aqueous solution is thereby transferred quickly and uniformly to the paper strip. The paper strip is separated from the blotter and heated, after dry ends have been torn off. Tests have shown that this method of application does not displace spots, and is superior to the most careful spraying of aqueous solutions. There is no appreciable transfer of carbohydrate to the blotting paper. The method is very useful with solutions that are obnoxious when sprayed ; for example, aqueous urea oxalate or sodium picrate. It does not succeed with most solutions in organic solvents, or with aqueous solutions of reagents that are adsorbed on the blotting paper, such as the chromium trioxide–permanganate–sulphuric acid reagent (below). Bowden and Maclagan (1954) used a similar technique to transfer an aqueous solution to a paper chromatogram, but did not separate the papers before development of the spots.

(iii) *Detection by Heating.*—Reducing sugars become visible after runs in sodium arsenite when the paper is dried and heated for a few minutes at 100–105 °C, mostly as orange or pale brown spots. L-Rhamnose and L-fucose give distinctly reddish spots, and melibiose is somewhat reddish. Under the ultraviolet lamp (" Chromatolite ", from Hanovia Ltd.) the spots show a strong yellow fluorescence, often with dark centres. The reaction seems to be general for reducing sugars, except tri- and tetra-*O*-methyl glucoses, and although not as sensitive as the reaction with aniline phosphate, it is sufficient for most purposes. The spots are permanent. If heating is stopped before the paper is completely dry, this development of colour is delayed, but not prevented, as visible brownish spots appear when the paper is stored, but the fluorescence is then only weak.

Reducing sugars on basic lead acetate papers also become visible when the paper is dried at 100–105 °C for some time, as pale yellow to brownish areas on a white background. The colours are rather weak, and tend to be strongest while the paper is hot. The reaction is useful for the location of moderate amounts of most reducing sugars, but rhamnose and methylated glucoses react only weakly. The spots do not fluoresce under ultraviolet light.

Some reducing sugars become faintly visible, as pale brown areas, when the paper is dried at 105 °C after runs in 0·1N sodium hydroxide.

(iv) *Aniline Salts.*—Most salts of aromatic amines used for the detection of reducing sugars on paper chromatograms are usable in the presence of each of the four electrolytes, but aniline phosphate (Hough, Jones, and Wadman 1950) seems to be the best, provided a suitable concentration is used. A solution of aniline (0·075M) plus phosphoric acid (0·15M) in *n*-butanol containing 12 per cent. water is sprayed on the paper, which is then heated for 3 min at 105 °C. In general, the aldohexoses give brown spots, the aldopentoses red or reddish brown spots, the ketohexoses pale brown spots, and the ketopentoses pale reddish spots. Detection of sucrose and raffinose may require longer heating. The sensitivity of the reaction is greatly improved by viewing the spots under the Chromatolite, as all, including those of ketoses (rather weak in daylight), sucrose, and raffinose, fluoresce strongly. The reaction of reducing sugars with aniline phosphate is less intense on basic lead acetate papers, all spots being more or less brown, and fluorescing more weakly under ultraviolet light. The reagent described is slightly supersaturated, and may need to be warmed to redissolve solids. The recipe of Bryson and Mitchell (1951) gives a grossly supersaturated solution.

Aniline picrate, in the form of a solution of aniline (0·1M) plus picric acid (0·2M) in acetone, has been used as a dip reagent for reducing sugars, sucrose, and raffinose (Frahn and Mills 1956*a*). When the paper is heated at 105 °C, the sugars appear as dark brown spots on a yellow background that slowly darkens. The reagent is not as sensitive as aniline phosphate, especially for ketoses, and the spots do not fluoresce under ultraviolet light. The picric acid is objectionable in stored papers. The lesser sensitivity may be an advantage when it is desired to detect the major components in a complex mixture of sugars that tend to streak, such as that from the acid " reversion " of a reducing sugar: aniline

picrate reveals the major concentrations as a series of nodes, whereas aniline phosphate gives an almost undifferentiated streak.

(v) *Urea Tartrate.*—Urea oxalate (Hirst, McGilvray, and Percival 1950) does not react satisfactorily with ketoses in the presence of borax. If the borax-containing paper is sprayed with urea oxalate, dried, and then dipped in a solution of salicylic acid in acetone and heated, better results are obtained. This suggests that a reagent is needed to displace the ketoses from the complexes with boric acid, and L-tartaric acid is the best of the reagents tried. An aqueous solution of urea (0·5M) plus tartaric acid (1M) is applied to the paper by the transfer process (above), and the paper is heated at 105 °C for 10 min. Ketohexoses, sucrose, and raffinose give strong blue or blue-green spots, and ketopentoses give pale greenish yellow spots, on a white background. Common aldoses give only weak grey or brown spots. D-Altrose, L-idose, D-allose, and D-talose react more strongly, but the spots have a reddish or brownish cast. L-Rhamnose and L-fucose give pale pink spots, and D-*glycero*-L-*gluco*heptose a bright pink spot. The more weakly reacting sugars may show a weak fluorescence under the Chromatolite. In practice, it is easy to distinguish ketohexoses from other reducing sugars. Fresh solution should be prepared weekly.

On sodium arsenite papers (dried without heating to the stage where reducing sugars are revealed), the aqueous urea tartrate solution gives results similar to those on borax papers. D-Altrose gives a faint blue-green spot, the aldo- and ketopentoses give pale yellow spots.

A more acidic solution is required for basic lead acetate papers: aqueous urea (0·5M) plus tartaric acid (2M). The sensitivity is less with this electrolyte. Ketohexoses give blue-green to blue-grey spots, the more reactive aldohexoses give yellow spots, and pentoses and common aldohexoses are scarcely detectable.

Although tartaric acid is added to liberate the ketoses from complexes with boric acid, the boric acid plays a part in the reaction, and if neither it nor arsenious acid is present the colours obtained with ketohexoses are weaker and only dull blue. For the detection of ketohexoses on paper chromatograms, a solution of urea (0·5M) plus tartaric acid (1M) in borax solution (0·05M) has been found satisfactory.

If papers run in 0·1N sodium hydroxide are dried carefully, without heating in the final stage of drying, it is possible to distinguish ketohexoses from other sugars in this electrolyte. The best reagent is a solution of urea (0·5M) plus tartaric acid (1·5M) in aqueous borax (0·05M).

Urea tartrate is best applied as an aqueous solution. A mixture of the same composition dissolved in *n*-butanol saturated with water gives inferior results, and experiments have shown that water is necessary for the reaction between urea tartrate and ketohexoses.

(vi) *Chromium Trioxide in Sulphuric Acid.*—A solution of chromium trioxide (1 per cent.) in aqueous 2N sulphuric acid is reduced more rapidly by most soluble polyhydroxy compounds than by cellulose (Frahn and Mills 1956*a*). The mixture must be sprayed on the paper. The sprayed paper is placed between glass plates (previously warmed), flanked by strips of moistened filter paper to prevent evaporation from the edges of the sprayed sheet. The assembly is then

stored at 35 °C for 30–45 min. Reactive compounds appear as pale areas within 10–15 min, but contrast is lost as continued oxidation of the cellulose bleaches the pale yellow background. This serves as a guide to the progress of the reaction. At the correct stage, as judged by spotting the paper (away from an edge) with a solution of diphenylcarbazide (1 per cent.) in ethanol, the paper is sprayed with the diphenylcarbazide solution. Oxidizable compounds are revealed as pale areas on the mauve background produced by the residual chromium trioxide. The colour soon fades, and for a permanent record the spots are ringed on the damp paper with a copying pencil, then the paper is exposed to ammonia vapour until neutralized, and dried.

This is a difficult technique, and considerable practice is needed to detect the more weakly reducing compounds. It is, however, the only method found suitable for detecting compounds other than reducing sugars in the presence of sodium arsenite. The arsenite is first oxidized to arsenate by spraying the dried paper with aqueous hydrogen peroxide (3 per cent.), then heating it until just dry. The paper is stored at room temperature overnight to allow residual hydrogen peroxide to decompose, heated for 1–2 min if any peroxide still remains, and then sprayed with the chromium trioxide in sulphuric acid. If hydrogen peroxide is not completely removed, the blue perchromic acid formed masks the reaction.

This reagent is useful in runs in basic lead acetate for detecting compounds that do not contain vicinal glycol groups. The precipitate of lead sulphate seems to protect the cellulose from oxidation by the chromium trioxide, and the contrast between the reducing areas and the background is good.

(vii) *Chromium Trioxide–Permanganate–Sulphuric Acid.*—Stock solutions of aqueous potassium permanganate (0·5 per cent.), and of chromium trioxide (0·25 per cent.) in 2N aqueous sulphuric acid, are mixed in the ratio 1 : 4 immediately before spraying; the reagent is therefore permanganate (0·1 per cent.) plus chromium trioxide (0·2 per cent.) in 1·6N sulphuric acid. In the original report (Frahn and Mills 1956*a*) the acid was erroneously stated to be 2N, which is in fact too high for the best results. The paper strip is sprayed, applying about 2 ml/100 cm^2, the unwetted areas are torn off, and the paper is stored between glass plates at room temperature. Highly reactive compounds are revealed in 3–6 min as pale yellowish green areas on the pink paper. About 5 min later, the pink colour fades, and is soon replaced by a uniform brown, on which the reducing compounds appear as pale areas. The brown background is stable for at least 1 hr, and during this time the less reactive compounds appear. The paper should be inspected at intervals, because spots due to low concentrations of highly reactive compounds may appear early and later merge into the background. For a permanent record, the paper is dipped into aqueous sodium carbonate, washed well, dried in the oven, and stored in the dark.

This reagent gives good results with reducing sugars (D-glucose reacts rather weakly), sugar alcohols, aldonic acids, cyclitols (except *scyllo*inositol), pentaerythritol, poly(vinyl alcohol), and many simple glycols. Some non-vicinal glycols, levoglucosan, and some glycopyranosides react only weakly. 2,3,6-Tri-*O*-methyl-D-glucose reacts strongly, and is a suitable non-migratory marker when

this spray is used. The reagent gives very good results on papers run in borax or sodium hydroxide, and is satisfactory on papers run in basic lead acetate; the lead gives pale yellow spots on a yellowish brown ground. It failed on papers run in sodium arsenite, even after oxidation of the arsenite with hydrogen peroxide. It has been used successfully on paper chromatograms developed in solvents containing *n*-butanol and pyridine, and thoroughly dried before spraying.

(viii) *Hydrogen Peroxide and Basic Lead Acetate.*—Dried papers from runs in basic lead acetate are sprayed with a freshly prepared solution of hydrogen peroxide (6 per cent.) in aqueous 1N ammonia to convert the lead to brown lead dioxide. The paper is left for about 3 min to let most of the ammonia evaporate, then sprayed with aqueous 50 per cent. acetic acid. Compounds containing vicinal glycol groupings are revealed almost immediately as pale areas on the brown background. The paper is kept for 15 min at room temperature, then washed in running water for 30 min, dried in the oven, and stored in the dark for a permanent record.

Sugar alcohols and most reducing sugars are detectable in low concentrations, but higher concentrations are needed for some simple glycols and cyclic compounds containing only *trans*-vicinal glycol groupings, such as levoglucosan and methyl D-glucopyranosides. 2,3,6-Tri-*O*-methyl-D-glucose is not detectable.

Somewhat less satisfactory results are obtained if the carefully dried basic lead acetate paper is dipped into a half-saturated solution of lead tetra-acetate in benzene (cf. Buchanan, Dekker, and Long 1950), and sprayed with a 2 per cent. solution of glacial acetic acid in 80 per cent. *n*-propyl alcohol after the benzene has evaporated. This method reveals sugar alcohols, and avoids the displacements that may occur during double spraying with aqueous solutions.

(ix) *Alkaline Silver Reagents.*—Any one of the commonly used alkaline silver reagents (Hough 1950; Trevelyan, Procter, and Harrison 1950; Cramer 1954, pp. 61, 71) serves for the detection of reducing sugars on papers run in borax, but the presence of borate greatly reduces the sensitivity of the reagents for non-reducing carbohydrates. The incorporation of pentaerythritol, which complexes well with borate and does not readily reduce silver oxide, corrects this deficiency. The dried borax paper is dipped into a solution of silver nitrate in acetone (Trevelyan, Procter, and Harrison 1950), dried (see below), and sprayed with a warm solution of sodium hydroxide (0·5N) plus pentaerythritol (4·5 per cent.) in ethanol. The sprayed paper is left at room temperature for 15 min, preferably in diffuse light, then washed with aqueous sodium thiosulphate (10 per cent.), left in running water for 30 min, and dried in the oven. The reducing sugars and other polyhydroxy compounds appear as intensely black stains on a cream or grey background. The intensity of the spots is shown on the arbitrary scale in Table 2.

Application of the sodium hydroxide and pentaerythritol in an alcoholic solution means that the mixture has to be warmed to dissolve them before spraying, but gives increased sensitivity compared with application as an aqueous solution, possibly because of a different physical form of the precipitated silver oxide.

TABLE 1

METHODS OF DETECTION AND NON-MIGRATING MARKERS

Markers shown in parentheses: (1) 2,3,6-tri-*O*-methyl-D-glucose, (2) 2,5-*O*-methylene-D-mannitol, (3) L-*threo*butane-2,3-diol, (4) pentaerythritol, (5) levoglucosan, (6) caffeine only suitable marker

Class	Borax	Sodium Arsenite	Sodium Hydroxide	Basic Lead Acetate
Reducing sugars	Aniline phosphate (1) Urea tartrate (6) $AgNO_3$ – NaOH – pentaerythritol (1,5) CrO_3–$KMnO_4$–H_2SO_4 (1)	Aniline phosphate (1) Urea tartrate (6) Heat; ultraviolet light (1) H_2O_2; CrO_3–H_2SO_4 (1)	Aniline phosphate (6) Urea tartrate+borax (6) $AgNO_3$ (6) CrO_3–$KMnO_4$–H_2SO_4 (3)	Aniline phosphate (1) Urea tartrate (6) Heat (1) H_2O_2–acetic acid (2,3) CrO_3–$KMnO_4$–H_2SO_4 (1,3,4) CrO_3–H_2SO_4 (1,4)
Sugar alcohols .. Glycosides .. Vicinal glycols ..	CrO_3–$KMnO_4$–H_2SO_4 (1) $AgNO_3$ – NaOH – pentaerythritol (1,5)	H_2O_2; CrO_3–H_2SO_4 (1)	$AgNO_3$ (6) CrO_3–$KMnO_4$–H_2SO_4 (3)	H_2O_2–acetic acid (2,3) CrO_3–$KMnO_4$–H_2SO_4 (1,3,4) CrO_3–H_2SO_4 (1,4)
Non-vicinal glycols Pentaerythritol	CrO_3–$KMnO_4$–H_2SO_4 (1) CrO_3–H_2SO_4 (5)	H_2O_2; CrO_3–H_2SO_4 (1)	CrO_3–$KMnO_4$–H_2SO_4 (3)	CrO_3–H_2SO_4 (1,4)

Some discrimination between different classes of compounds may be achieved as follows. After the borax paper is dipped into the silver nitrate in acetone and dried in air, it is warmed gently for 1–2 min under a Phillips HPW–125W ultraviolet lamp in a darkened room (the Chromatolite does not provide sufficient heat). The following aldoses are seen as yellow to green fluorescent spots: unsubstituted aldopentoses and aldohexoses, cellobiose, lactose, and maltose (fairly strong); 2,3,6- and 2,4,6-tri-*O*-methyl-D-glucose (as 1M solutions) and melibiose (weak). Ketohexoses, 3-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-glucose, turanose, sucrose, and raffinose are not detectable. The paper is now steamed over boiling water for 5 min, whereupon the unsubstituted aldopentoses and aldohexoses, and reducing aldose disaccharides become visible as yellow to brown spots. Ketohexoses, the foregoing methylated D-glucoses, sucrose, and raffinose remain invisible. The paper is now further exposed to the ultraviolet lamp; the unsubstituted aldoses and reducing disaccharides no longer fluoresce, but a fluorescence is now seen with ketohexoses, 3-*O*-methyl-D-glucose, 2,3,6- and 2,4,6-tri-*O*-methyl-D-glucose, methyl α-D-mannopyranoside, D-arabitol, D-mannitol, D-glucitol, dulcitol, and 2-amino-2-deoxy-D-glucitol, being strongest for the last two. Some compounds, in particular the ketopentoses, have not yet been submitted to this procedure.

After runs in 0·1N sodium hydroxide, reducing sugars may be detected by dipping the dried paper into the solution of silver nitrate in acetone; they appear as brown to black areas as the acetone evaporates. Sugar alcohols react only weakly, but the results are improved by spraying with ethanol. After reaction for 30 min in diffuse light the paper is fixed in sodium thiosulphate in the usual way.

In general, the alkaline silver reagents will not detect compounds that lack vicinal glycol groupings (cf. Ivanov and Sokova 1948). They are not suitable for papers run in sodium arsenite or basic lead acetate.

(x) *Summary.*—Methods of detection suitable for the several classes of compounds in the different electrolytes are listed in Table 1, approximately in order of usefulness.

(*f*) *Markers and Reference Substances*

(i) *Non-Migrating Markers.*—A single non-migrating marker equally suitable for the correction for electro-osmosis in all electrolytes has not been found. It should be readily detectable, either visually or by the reagents used for the carbohydrates.

2,3,6-Tri-*O*-methyl-D-glucose, 2,4,6-tri-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose do not migrate in borax, sodium arsenite, or basic lead acetate, and if applied in 1M solutions are easily detected by the aniline salt reagents for aldoses and by alkaline silver nitrate. 2,3,6-Tri-*O*-methyl-D-glucose is the most useful, as it is also revealed by the chromium trioxide–sulphuric acid and chromium trioxide–permanganate–sulphuric acid mixtures. None of these methylated D-glucoses is revealed by the reagent for ketoses, or by the hydrogen peroxide spray on basic lead acetate papers. They all migrate in sodium hydroxide.

For solutions more alkaline than pH 3, Crestfield and Allen (1955) recommended caffeine as a generally useful non-migrating marker that has the advantage of being visible under the Chromatolite and other lamps emitting the 2537 Å mercury line. The fluorescence is weak on basic lead acetate papers, and caffeine seems to have a slight migration in all four electrolytes relative to the above carbohydrate markers, but it is very useful for all but the most accurate work.

Several compounds containing vicinal glycol groupings do not migrate in basic lead acetate, and may be used as markers when using the hydrogen peroxide spray ; the best are 2,5-*O*-methylene-D-mannitol, 1,2-*O*-*iso*propylidene-D-glucose, and L-*threo*butane-2,3-diol. D-Mannitol, previously suggested as a marker in 0·1N sodium hydroxide (Frahn and Mills 1956*a*), has now been found to migrate in this electrolyte, but L-*threo*butane-2,3-diol does not, and may be used in conjunction with the chromium trioxide–permanganate–sulphuric acid reagent. Levoglucosan does not migrate in borax, and reacts well with the silver nitrate–sodium hydroxide–pentaerythritol reagent.

(ii) *Reference Carbohydrates.*—No single substance is suitable as a standard for the comparison of rates of migration in all electrolytes. D-Glucose has been widely used as a standard in borate buffers, rates of migration relative to it being listed as " M_G values " (Foster 1952). M_G values cannot be measured accurately for sodium arsenite or basic lead acetate, in which glucose has a very low rate of migration, and it is suggested that D-ribose be used as standard substance in these electrolytes and in 0·1N sodium hydroxide, because it has nearly the highest rate in all three. Rates of migration calculated relative to D-ribose are shown as " M_R values " in Table 2. Ribose reacts readily with all reagents except that for ketoses. It would be a suitable reference substance in borate solutions, too, because it retains a high rate of migration, without excessive streaking, when the pH or concentration of borate is decreased (Consden and Stanier 1952 ; and our unpublished data).

(iii) *A General Standard of Rate.*—Although it is convenient to use a standard carbohydrate with which to compare the rates of migration of others, the method has disadvantages. Different electrolytes may require different standards, and the rate of migration of the standard in a given electrolyte will change with changes in pH or concentration of electrolyte. Angyal and McHugh (1957) found that D-glucose migrates more slowly, and streaks, in very dilute borate solutions. The full information required for theoretical discussion of the influence of the nature of the electrolyte, its concentration, and its pH on complex formation can only be obtained by measuring the absolute mobilities of carbohydrates (difficult with the present apparatus), or by using as standard of rate a compound that has practically the same mobility under all conditions used.

The nitrobenzene-*p*-sulphonate ion seems to be a suitable standard of reference for all anionic complexes. It moves at a convenient rate, is easily detected in ultraviolet light, and is inert to carbohydrates and the common electrolytes. The strength of nitrobenzene-*p*-sulphonic acid ensures its complete ionization in dilute solution at all usable pH values, therefore the mobility of the standard is independent of pH. It is reasonable to assume that changes in temperature, viscosity, or ionic strength of electrolyte will alter the mobility of

the nitrobenzene-*p*-sulphonate ion and of the carbohydrate complexes to about the same extent. Experiments performed to date confirm these expectations.

It is therefore proposed that rates of migration of carbohydrates as anionic complexes should ultimately be compared with the rate of migration of the nitrobenzene-*p*-sulphonate ion under the same conditions, and that the ratios so calculated should be designated " M_N values ". The M_N value of D-glucose in 0·05M borax is 0·93, and of D-ribose in sodium arsenite at pH 9·6, 0·34. It is more difficult to measure M_N values accurately in sodium hydroxide, as the nitrobenzene-*p*-sulphonate ion moves far ahead of all carbohydrates, and swelling of the paper makes it difficult to ensure uniform movement throughout the whole of the strip.

No attempt has been made to find a cationic standard to replace D-ribose in ionophoresis in basic lead acetate.

(iv) *Mixtures of Markers.*—A suitable non-migrating marker and a standard of rate may be combined in the one solution. For borax and sodium arsenite, the most useful combination is aqueous caffeine (1·4 per cent.) plus potassium nitrobenzene-*p*-sulphonate (0·5 per cent.), both being detected under the Chromatolite. For sodium hydroxide and basic lead acetate, the most useful mixture is caffeine (1·4 per cent.) plus D-ribose (1·8 per cent.). For highly accurate work, caffeine may be replaced by a non-migrating marker suited to the method of detection used; a selection of such markers is given in Table 1. If it is necessary for efficient detection, the concentration of the non-migrating carbohydrates may be greater than 0·1M, for example, 0·5–1·0M for the tri-*O*-methyl glucose and 2,5-*O*-methylenemannitol.

(*g*) *Measurement of Spots*

Mostly the spots are nearly circular, and the distance to the centre of the spot is measured. If markedly elongated spots are obtained (mainly in basic lead acetate), the leading edge is nearly always sharply defined, and in these cases the distance is measured to the centre of curvature of the leading edge; the shape of the spot is noted in Table 2. This procedure has been adopted because it is believed that the elongation is due mainly to partial adsorption of the carbohydrate on the paper (see below), or an unduly high concentration; and perhaps in part to the relatively slow mutarotation of some reducing sugars at pH 6·8, which could allow part of the sugar to lag during migration; if any of these causes apply, the leading edge will provide the best criterion of the true rate of migration.

(*h*) *Adsorption of Carbohydrates on the Paper*

Only one compound, *scyllo*inositol in basic lead acetate, seemed to be very strongly and irreversibly adsorbed on the paper support, appearing as a small, concentrated spot at the site of application. The marked elongation or streaking observed with several other compounds in basic lead acetate may be evidence of partial adsorption on the paper. Lead ions seem to be more strongly adsorbed on filter paper than the other electrolytes used, and complexing of soluble carbohydrate with bound lead ions may be the source of retardation. On this basis, non-complexing soluble carbohydrates should not be retarded, but *scyllo*inositol,

for which complexing is unlikely, is the most strongly adsorbed of all compounds tested.

Partial adsorption of some sugars on paper in the presence of basic lead acetate was demonstrated by wetting a paper strip with the electrolyte at one end, lightly blotting it, spotting test solutions across the wetted portion, then clamping the paper between glass plates, dipping the wetted end into electrolyte, and allowing this to advance into the dry region by capillarity for about 1 hr. The following relative movements of sugars were observed: D-glucose, 100; L-sorbose, 100; D-ribose, 83; D-fructose, 89; D-mannose and L-rhamnose, extensive streaking, with the visible front well behind that of D-glucose. The last four sugars are definitely retarded by adsorption, and the first two may be to a lesser extent. The rapidity of the capillary rise, relative to the electro-osmotic movement or migration in ionophoresis, may have magnified the effect.

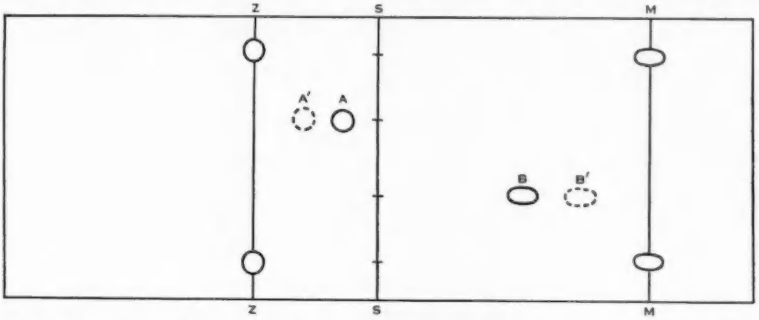

Fig. 1.—Effect of partial adsorption of compounds on paper support (hypothetical case). *A*,*B*: Observed positions of compounds that are retarded by adsorption; *A'*,*B'*: positions they would occupy if no adsorption occurred. Direction of migration is from left to right; of electro-osmotic flow, from right to left. *SS*, Line of application; *ZZ*, line of true zero migration; *MM*, positions of standards of rate.

Since electro-osmosis is opposed to the direction of migration of the electrically charged carbohydrate complexes in the four electrolytes, there is a region, between the true zero line and the starting line, in which retardation of movement by adsorption will cause an apparent *increase* in mobility relative to compounds that are not adsorbed (see Fig. 1). Non-complexing compounds could therefore appear to be migrating. The approximate *M* value at the site of application is recorded for each electrolyte in Table 2; *M* values less than this could be greater than the true values, and conversely, *M* values greater than that at the starting line could be less than the true values, if adsorption on paper is occurring.

No allowance for the possible effects of adsorption is made in the following discussion. It is assumed to be unimportant for carbohydrates of low molecular weight in borax and sodium arsenite. The proven adsorption on paper in the presence of basic lead acetate may lead to results somewhat different from those in Table 2 if a different grade of paper is used, or the electrolyte has a slightly different composition.

(*j*) *Summary of Results*

Results obtained to date in the four electrolytes are shown in Table 2. Relative rates of migration have been multiplied by 100 for clarity. M_N values are not shown, but may be calculated for borax and sodium arsenite from the data in Section II (*f*) (iii). In addition to compounds mentioned in Section II (*a*), the following were generally applied at concentrations greater than 0·1M: most

TABLE 2

RELATIVE RATES OF MIGRATION IN PRINCIPAL ELECTROLYTES

Ninety-minute runs at 20–25 V/cm, with room temperature 20–25 °C and cooling water 18–20 °C; applied solutions 0·1M unless specified in text

Compounds Tested	Borax 0·05M (10^2M_G)	Reaction with $AgNO_3$*	Sodium Arsenite pH 9·6 (10^2M_R)	Sodium Hydroxide 0·1N (10^2M_R)	Basic Lead Acetate (10^2M_R)
Reducing sugars					
L-Arabinose	91	3	30	79	7
D-Lyxose	71	—	42	97	30
D-Ribose	75	3	100	100	100
D-Xylose	101	3	17	93	8
L-Fucose	83*E*†	3	22	60	6
L-Rhamnose	49	3	32	88	28
D-Allose	83	3	75	68	33
D-Altrose	97	3	77	96	10
D-Galactose	93	3	28	65	10
D-Glucose	100	3	16	80	6
D-Gulose	82	—	53	70	31
L-Idose	102	3	115	96	42
D-Mannose	69	3	35	84	41
D-Talose	87	3	119	103	110
D-*erythro*Pentulose	90	—	209	—	73
D-*threo*Pentulose	75	—	194	—	41
D-Fructose	89	3	75	89	22
D-Psicose	76	2	188	125	91*S*†
L-Sorbose	97	3	73	88	16
D-Tagatose	95	2	103	82	65
D-*glycero*-L-*gluco*Heptose	104	3	23	72	7
3-*O*-Methyl-D-glucose	76	2	13	77	0
2,3,6-Tri-*O*-methyl-D-glucose	0‡	1	0‡	71	0‡
2,4,6-Tri-*O*-methyl-D-glucose	0‡	0–1	0‡	66	0‡
3,5,6-Tri-*O*-methyl-D-glucose	65	—	134	—	8
Tetra-*O*-methyl-D-glucopyranose	0‡	1	0‡	74	0‡
Cellobiose	22	3	15	68	10
Lactose	37	3	24	61	13
Maltose	30	3	15	68	7
Melibiose	77	2	32	62	10
Turanose	64	2	30	79	7

* Method of Section II (*e*) (ix): 3, strong reduction; 2, moderate; 1, weak.

† *E*, markedly elongated spot; *S*, extensive streaking.

‡ Non-migrating by definition.

TABLE 2 (*Continued*)

Compounds Tested	Borax 0·05M (10^2M_G)	Reaction with $AgNO_3$*	Sodium Arsenite pH 9·6 (10^2M_R)	Sodium Hydroxide 0·1N (10^2M_R)	Basic Lead Acetate (10^2M_R)
Glycosides					
Sucrose	16	2	14	29	4
Raffinose	26	2	25	30	7
Methyl α-D-altropyranoside	58	1	—	7	1
Methyl α-D-galactopyranoside	35	1	19	6	5
Methyl β-D-galactopyranoside	34	1	19	7	5
Methyl α-D-glucopyranoside	10	2	9	9	1
Methyl β-D-glucopyranoside	15	3	6	9	3
Methyl α-D-mannopyranoside	39	2	17	9	0
Phenyl β-D-glucopyranoside	15	3	12	9	4
Sugar alcohols					
Glycerol	49	2	24	0	3
Erythritol	75	1	53	3	3
L-Threitol	75	1	96	3	11
D-Arabitol	87	2	124	7	14
Ribitol	85	3	76	10	4
Xylitol	79	3	155	3	25
Allitol	90	1	92	23	9
Dulcitol	97	3	145	8	32
D-Glucitol	83	3	161	11	47
L-Iditol	81	3	173	7	57
D-Mannitol	91	3	130	12	23
D-Talitol	89	1	138	16	17
D-*glycero*-D-*altro*Heptitol	92	2	144	44	27
D-*glycero*-D-*galacto*Heptitol	98	1	140	11	51*E*†
D-*glycero*-D-*gluco*Heptitol	88	2	171	30	53*E*†
D-*glycero*-L-*gluco*Heptitol	95	1	176	17	59*E*
D-*glycero*-D-*ido*Heptitol	85	2	168	20	71*E*
D-*glycero*-D-*talo*Heptitol	93	1	140	24	34
meso-*glycero*-*allo*Heptitol	95	2	100	54	11
meso-*glycero*-*gulo*Heptitol	85	2	160	27	72*E*
meso-*glycero*-*ido*Heptitol	78	1	182	17	79*E*
Other glycols					
Ethylene glycol	11	1	3	—	0
Propane-1,2-diol	16	1	5	0	0
Propane-1,3-diol	5	0	0	—	—
Butane-1,3-diol	10	0	0	—	—
*erythro*Butane-2,3-diol	13	1	6	0	0
L-*threo*Butane-2,3-diol	51	1	33	0‡	0
Butane-1,4-diol	0	0	0	—	—
*erythro*Pentane-2,4-diol	18	0–1	0	0	0
*threo*Pentane-2,4-diol	0	0–1	0	—	—

* Method of Section II (*e*) (ix): 3, strong reduction; 2, moderate; 1, weak.
† *E*, markedly elongated spot; *S*, extensive streaking.
‡ Non-migrating by definition.

TABLE 2 (*Continued*)

Compounds Tested	Borax 0·05M (10^3M_G)	Reaction with $AgNO_3$*	Sodium Arsenite pH 9·6 (10^3M_R)	Sodium Hydroxide 0·1N (10^3M_R)	Basic Lead Acetate (10^3M_R)
Other glycols—*Continued*					
Pentane-1,5-diol	0	0	0	—	—
2-Methylpentane-1,3-diol	8; 24	0	0	—	—
Pentaerythritol	85	0	21	12	0
Poly(vinyl alcohol)	52*E*	0	68*E*	11*E*	0
*cis-cyclo*Hexane-1,2-diol	6	1	9	0	0
*trans-cyclo*Hexane-1,2-diol	0	1	2	—	0
Cyclitols					
*allo*Inositol	85	2	50	9	62
*cis*Inositol	79	1	40	9	116
*epi*Inositol	76	1	29	5	74*S*
(—)-Inositol	59	2	23	3	20
*muco*Inositol	97	1	36	15	41
*myo*Inositol	49*E*	2	16	2	75*E*
*neo*Inositol	59	2	43	1	64
*scyllo*Inositol	2	1	7	3	Adsorbed
3-*O*-Methyl-(+)-inositol	59	1	35	—	2
2-*O*-Methyl-(—)-inositol	24	2	27	—	14
1-*O*-Methyl*myo*inositol	11	—	20	—	26
5-*O*-Methyl*myo*inositol	15	—	25	—	22
Miscellaneous carbohydrates					
Levoglucosan	0	3	0	5	8
1,2-*O-iso*Propylidene-D-glucose	71	1	25	31	0
1,2-*O-cyclo*Hexylidene-D-mannitol	63	1	81	—	3
3,4-*O-iso*Propylidene-D-mannitol	61	1	44	—	0
3,4-*O*-Methylene-D-mannitol	66	1	47	—	2
2,5-*O*-Methylene-D-mannitol	62	1	28	—	0
2,4-*O*-Methylene-D-glucitol	58	1	33	—	0
2-Amino-2-deoxy-D-glucitol	47	2	51	8	116
Caffeine (*M* value)	2	—	6	4	4
M value at site of application	25	—	75	70	40
Mean migration (cm) of standard in 90 min at 25 V/cm	20	—	8	13	11

* Method of Section II (*e*) (ix): 3, strong reduction; 2, moderate; 1, weak.

simple glycols (0·2–0·4M), glycopyranosides (0·2–0·4M), and non-migrating carbohydrate markers (up to 1M).

The figures in Table 2 have been selected from closely concordant repetitions. Some of the figures were obtained before the procedure had evolved to the form recommended here, but it is believed that the overall consistency of the data is good. Ideally, any compounds found in different runs to have similar relative

rates of migration should be compared in adjoining lanes in further runs, but because of the number and variety of compounds examined this has not been possible in all cases. The accuracy of the data should be sufficient to show whether a given mixture can be separated.

The present data supersede those in the preliminary communication (Frahn and Mills 1956*a*), which in several cases were slightly in error due to inadequate methods used in running, drying, and spraying papers. The position of dulcitol relative to other hexitols in sodium arsenite was wrongly reported, because of difficulty at that time in locating dulcitol in the presence of sodium arsenite. As was pointed out by Gross (1956), the position of pentaerythritol relative to D-glucitol in borax was also incorrect.

(*k*) *Results under Non-Standard Conditions*

The rate of migration of a substance in each electrolyte generally seems to be independent of the concentration of the test solution. Several compounds have been run in 0·05M borax at applied concentrations ranging from 1·0 to 0·06M. The M_G values for D-mannitol, pentaerythritol, and L-*threo*butane-2,3-diol are the same at all concentrations. The reducing sugars D-glucose and D-fructose appear to migrate slightly more rapidly at the high concentrations, but the spots are markedly elongated and it is difficult to determine the point from which the measurements should be made ; if the radius of curvature of the leading edge is taken, the effect of changed concentration is very small.

There are indications that the M_R values of some compounds in basic lead acetate alter slightly at high applied concentrations, but again marked elongation of the spots causes uncertainty in the measurements. The streaking may be due to increased adsorption of carbohydrate on the paper at higher concentrations, or, in the case of reducing sugars, to slowness of mutarotation at pH 6·8, which could result in trailing of that part of the sugar not initially combined with the lead ions. The M_R value of L-iditol increases by 10 per cent. when the concentration of the applied solution is reduced from 0·1 to 0·02M.

When it is necessary to run solutions of markedly different concentrations, best results seem to be obtained by using sensitive methods of detection, which reveal the full extent of the spots and permit the centre of curvature of the leading edge to be accurately located. Excessively concentrated solutions should be avoided because of the possible variations in M values, and the risk of obscuring slower moving, minor components by the trailing of the main spot.

The presence of moderate concentrations of salts that do not react with the electrolyte or with the polyhydroxy compounds seems to have no effect on the rate of migration of the carbohydrates. Test solutions should be neutralized before application, and must be free of boric acid for runs in electrolytes other than borax. Identical results were obtained with 0·1M solutions of carbohydrates prepared in water and in the electrolytes used for the runs.

The order of relative mobilities was not altered by performing the ionophoresis at a different potential gradient or for a different length of time, with the possible exception of a few compounds run in basic lead acetate. For example, after a run of 3 hr at 13 V/cm in this electrolyte instead of 1·5 hr at 24 V/cm,

the M_R value of D-altrose was 0·15 instead of 0·10. Runs in basic lead acetate or sodium hydroxide should be limited to 1·5 to 2 hr because of the extensive destruction of reducing sugars that occurs in these solutions. D-Psicose could not be detected after a 3 hr run in lead. In general, excessively long runs may not confer any advantage, as the increased linear separation of spots is counteracted by greater diffusion and difficulties in detection, but runs in sodium arsenite may be extended to 3 hr to improve the separation of the slower reducing sugars, as the spots are normally small and regular.

Very high potential gradients have not been used with electrolytes other than borax (Gross 1955). It is likely that their use with basic lead acetate would increase the tendency to streaking already observed for the faster-moving compounds. High gradients probably would be advantageous with sodium arsenite.

(l) Other Electrolytes Tested

No significant ionophoretic migration of the common reducing sugars and hexitols was found in aqueous solutions of the following neutral salts, each 0·2M with respect to the cation: lead acetate, copper sulphate, zinc sulphate, ferrous ammonium sulphate, and calcium chloride. Other electrolytes not affording electrically charged complexes with these common carbohydrates include hydrochloric acid, potassium dichromate, and ferric chloride adjusted to pH 3 (all 0·1M); aluminium sulphate, magnesium sulphate, and sodium sulphate (all 0·05M); and potassium antimonyl tartrate (0·2M). D-Ribose may have moved slightly in some of them. Solutions of zinc sulphate or copper sulphate in excess aqueous ammonia were also ineffective as electrolytes.

Aqueous sodium carbonate (0·1M) at pH 10 did not cause appreciable migration of reducing sugars or other non-acidic compounds. This suggests that the observed migrations of non-acidic polyhydroxy compounds in borax and sodium arsenite are due only to the formation of complexes, and not in part to the alkalinity of the electrolytes. The migration of sugars in 0·01N sodium hydroxide was far less than in the 0·1N sodium hydroxide described above. A fairly rapid movement of several carbohydrates was observed in sodium aluminate (approx. 0·05M $Al(OH)_3$ in 0·1N NaOH), but the solution was very alkaline and it was not further examined after the finding that aqueous alkali alone permitted migration.

A solution of sodium arsenite nominally 0·2M NaH_2AsO_3 was prepared, but it had an inconveniently high pH (11·0), and did not offer significant advantages over the solution of pH 9·6 in separations of reducing sugars. No appreciable migration of sugars could be detected in a solution of sodium arsenite at pH 8·5. No migration was found in solutions of sodium arsenate or arsenic acid over a wide range of pH. Sodium phosphate has been reported not to cause migration of carbohydrates (Consden and Stanier 1952).

As already reported (Frahn and Mills 1956*a*), a preliminary study showed significant migrations, as anions, of reducing sugars and sugar alcohols in sodium tungstate, ammonium molybdate, and sodium metavanadate, but these systems have not been further examined. The solutions contained 0·2 g-atom of heavy

metal per litre, and the optimum pH for migration was, approximately, 6·2 for tungstate, 5·6 for molybdate, and 8·6 for metavanadate. Several reducing sugars, especially D-ribose, migrated rapidly in tungstate and molybdate, and slowly in metavanadate, but extensive streaking occurred. Mannitol, dulcitol, and glucitol migrated rapidly in tungstate and molybdate, without being separated, but glucitol could be separated from the other two in metavanadate. The carbohydrates became visible on drying the paper after runs in metavanadate; with the other electrolytes, reducing sugars were revealed by spraying with aniline salts, and sugar alcohols by alkaline permanganate. When these easily reducible electrolytes are used, connection should be made through a salt bridge to protect them from electrode reactions.

III. Discussion

(*a*) *Efficiency of Separation and Detection*

The comparisons presented in Table 2 show that the use of sodium arsenite, basic lead acetate, and sodium hydroxide as electrolytes greatly increases the scope and practical utility of paper ionophoresis of carbohydrates. Separations of mixtures of reducing sugars or sugar alcohols are more easily achieved than when borate buffers only are used.

The 21 unsubstituted reducing monosaccharides listed in Table 2 have M_G values in borax ranging from 0·49 to 1·04, with 15 in the range 0·82 to 1·04, whereas the range in M_R values for the 21 sugars is 0·16 to 2·09 in sodium arsenite and 0·06 to 1·10 in basic lead acetate, with better distribution of individual sugars throughout the intervals. More complex mixtures may therefore be separated in the latter two electrolytes, notwithstanding the smaller average distances of migration of the faster sugars. Sodium arsenite has the additional advantages of generally affording small, regular spots and needing no application of reagents to reveal the spots. It is the electrolyte of first choice for the examination of mixtures of reducing sugars.

When the commonly occurring pentoses, hexoses, and 6-deoxyhexoses are considered, it is found that (i) mannose and rhamnose are slow in borax, but fairly rapid in basic lead acetate; (ii) xylose, glucose, arabinose, and galactose migrate very rapidly in borax, but very slowly in basic lead acetate; (iii) galactose and glucose may be separated more easily in sodium arsenite than in borax, and the order of mobilities is different in the two electrolytes; (iv) fructose and sorbose have characteristically high mobilities in sodium arsenite. By taking advantage of the differences in order of mobilities in the several electrolytes, it should be possible to identify the components of mixtures of common sugars without recourse to paper chromatography, with a considerable saving in time. Sodium hydroxide as electrolyte performs the special function of separating the pairs glucose–xylose and galactose–arabinose, which cannot be resolved in the other electrolytes. Paper chromatography would probably be needed for the detection of small amounts of glucose or galactose in the presence of large amounts of xylose or arabinose, respectively, but ionophoresis is generally preferable. For example, the best chromatographic separation of galactose and glucose reported by Chargaff, Levine, and Green (1948) involved a difference in R_F values of only

12 per cent. (0·33 and 0·37, respectively), but the ratio of mobilities in sodium arsenite is 1·75 : 1.

The separation of sugar alcohols is very poor in borax, the range in M_G values for all isomers containing four to seven carbon atoms being only 0·75 to 0·98. The range in M_R values in sodium arsenite and basic lead acetate is much greater, and isomers are usually well separated. Nevertheless, the separation of mixtures of sugar alcohols is more difficult than separation of reducing sugars : the order in sodium arsenite and basic lead acetate is nearly the same, sodium arsenite poses formidable problems of location at low concentrations, and many of the faster-moving alcohols give elongated spots in basic lead acetate. The identification of individual sugar alcohols by paper ionophoresis should be easy, if known compounds are available for comparison. Several of the heptitols show useful rates of migration in sodium hydroxide, in approximately the reverse order to that in the other electrolytes. Borax and basic lead acetate each permit useful separations of inositols, and the order is different in the two electrolytes.

Borax is the best electrolyte for separations of stereoisomeric compounds of fixed cyclic structure, such as glycosides (cf. Foster and Stacey 1955 ; Foster 1957*a*, 1957*b*), or containing few hydroxyl groups, such as glycols. Most compounds in these classes have small or zero migrations in the other electrolytes.

Paper ionophoresis in each electrolyte has the advantage over paper chromatography in being generally unaffected by the presence of moderate concentrations of salts or other inert impurities in the carbohydrates. The important restrictions are that the impurities should not cause a precipitation of solid that adsorbs carbohydrates, and that borates must be absent from samples run in the other electrolytes. The complexing ability of borate is so great that traces of it may alter the rate of migration in other electrolytes. It was found that a solution of D-glucose in borax applied to a run in sodium arsenite gave two spots for reducing sugar, one in the normal position for glucose in arsenite, the other approximately in the position found for glucose run in borax, with a streak between. Part of the glucose–borate complex evidently survived for 90 min while moving through the solution of sodium arsenite. This behaviour could possibly be used as an additional aid to the identification of some carbohydrates.

When compounds that do not give elongated spots are applied at approximately 0·1M concentration, the presence of two components in a mixture may generally be detected if the centres of the spots lie 1 cm apart ; this corresponds to a difference of about 0·05 in M_G values in borax, and of about 0·1 in M_R values in sodium arsenite or basic lead acetate, under the standard conditions. A lesser separation may be sufficient for reducing sugars in sodium arsenite, and for sugars that give different colours with aniline phosphate after runs in borax (e.g. pentose and hexose). A clear indication of the non-identity of two compounds should be obtainable if the distances of migration differ by 0·5 cm, provided equal concentrations of the two are applied alternately across the paper. Reducing sugars in sodium hydroxide afford spots more diffuse than in the other electrolytes, and greater linear separations are needed if mixtures are to be resolved with confidence.

The methods of detection described are believed to be adequate for most purposes, except for the location of non-reducing carbohydrates after runs in sodium arsenite, for which a reliable procedure is urgently needed. The addition of a complexing agent for boric acid, to improve the sensitivity of detecting agents in the presence of borax, illustrated for the alkaline silver reagent and the urea reagent for ketoses, may prove to be useful with other reagents. Detecting agents other than those listed in Section II have not been tested exhaustively, but the periodate–permanganate reagent of Lemieux and Bauer (1954) seemed to be satisfactory in the presence of borax, whereas the sodium 3,5-dinitrosalicylate reagent for reducing sugars (Jeanes, Wise, and Dimler 1951) proved to be relatively insensitive. The chromium trioxide–permanganate–sulphuric acid reagent is recommended for the general detection of oxidizable organic compounds. Urea tartrate plus borax seems to be better than reagents hitherto used for detecting ketohexoses. It is significant that D-*glycero*-L-*gluco*heptose should give a pink spot with urea tartrate, as Green (1958) subsequently reported that several aldoheptoses give pink spots with urea phosphate. 6-Deoxyaldohexoses react differently with the two spray reagents.

(*b*) *Relations between Configuration and Rate of Migration*

The following comments are intended to illustrate potential applications of paper ionophoresis to problems of carbohydrate stereochemistry, a detailed discussion of the nature of the migrating species being deferred. It will be assumed that migration (except in sodium hydroxide) occurs only when at least two hydroxyl groups in a molecule of polyhydroxy compound are united with the electrolyte ion as a cyclic complex, that the formation and breakdown of the complexes is reversible and very rapid, and that the relative mobilities of similar compounds are approximate measures of the equilibrium constants for formation of the complexes (cf. Angyal and McHugh 1957). Small differences in relative mobility may not be significant because of the many factors that can affect ionic migration.

In borax, effective separation of stereoisomers is often possible when only one pair of hydroxyl groups is available, as in simple glycols, or when the structure permits complexing only at one point, as in many glycosides (Foster and Stacey 1955; Foster 1957*a*, 1957*b*). Either five-membered or six-membered cyclic complexes are possible, and the equilibrium apparently is often greatly in favour of the complex, even for glycols. Five-membered rings seem to be somewhat favoured over six-membered. The relative ease of formation of cyclic borate complexes from the stereoisomeric butane-2,3-diols and pentane-2,4-diols is the same as the relative ease of formation of cyclic acetals, and it is highly probable that cyclic borate and cyclic acetal (more specifically, cyclic ketal) ring systems are sterically very similar. Foster has made a similar assumption in discussing cyclic borate complexes of glycosides. The mobilities of simple glycols and polyhydroxy compounds of fixed cyclic structure may therefore be interpreted by using the rules that apply to the formation of cyclic acetals (Mills 1955). In particular, paper ionophoresis in borax is a promising method for separating

and assigning configurations to *erythro-* and *threo*-forms of acyclic αβ- and αγ-glycols.

The great complexing ability of borax, and the readiness with which it forms five-membered or six-membered cyclic complexes, and also "tridentate" complexes (Angyal and McHugh 1957), explain the generally poor separation of isomeric sugar alcohols, and reducing sugars, in borax. These have mobile structures, and each isomer can provide several structural features favourable for complexing, therefore they are virtually "saturated" with complex borate groups in solution, and have similar rates of migration. There is evidence (unpublished) that many of them are combined with more than one molecule of boric acid during migration.

Sodium arsenite and basic lead acetate form relatively weak complexes in solution, and little migration is observed for most glycols and glycosides, especially in lead. These electrolytes show marked selectivity in the separation of reducing sugars and sugar alcohols because complexing, when it occurs, is confined to the most favoured sites. The behaviour of sugar alcohols in basic lead acetate shows that formation of five-membered cyclic complexes only is the dominant process. The structure most favourable for the formation of a five-membered complex is a pair of hydroxyl groups of *threo* configuration, and the mobilities of sugar alcohols of *ribo*, *allo*, and *meso-glycero-allo* configurations, which contain no *threo* groups, are uniformly low. Mobility increases with an increase in the number of *threo* groups, reaching a maximum for the *xylo*, *ido*, and *meso-glycero-ido* configurations. The correlation is close enough to show that ionophoresis in basic lead acetate may be a valuable procedure for stereochemical investigations into the structure of acyclic polyols, for which borax is unsuitable. There is no evidence for the formation of six-membered complexes in lead.

Sugar alcohols show the same general trend of mobilities in sodium arsenite as in basic lead acetate, but there are some variations in the order of heptitols, and the migrations in arsenite are greater, especially for those with *ribo* and *allo* configurations. Sodium arsenite seems to have greater complexing power, but the predominant type of complex is again that with five-membered rings, although some six-membered, and also tridentate, complexes may be present.

The behaviour of the inositols in arsenite and basic lead acetate shows a fairly good correlation between the mobility and the number of pairs of adjacent *cis*-hydroxyl groups, more particularly for lead, and both electrolytes therefore readily afford complexes in which a five-membered ring is fused through a *cis*-ring junction to a six-membered ring.

For reagents that preferentially afford five-membered cyclic complexes, the most favourable situation for complexing is usually provided by a pair of *cis*-hydroxyl groups attached to a five-membered ring (e.g. *cis-cyclo*pentane-1,2-diol). Reducing sugars that have a high content of furanose form through conformational instability of the pyranose form, or are held in the furanose form by substitution, should therefore show high mobilities in sodium arsenite and basic lead acetate, provided two adjacent *cis*-hydroxyl groups are free. Among the aldoses, this expectation is realized fairly completely in sodium arsenite, the conformationally stable glucose and xylose being the slowest, and the conformationally unstable

ribose, idose, talose, and altrose the fastest. The agreement is less satisfactory for basic lead acetate: the conformationally stable sugars are slow, but only talose and ribose are exceptionally fast, and 3,5,6-tri-*O*-methyl-D-glucofuranose is surprisingly slow. Possibly the total number of pairs of *cis*-hydroxyl groups in the pyranose and furanose form, rather than a single, particularly favourable pair of *cis*-hydroxyl groups, is the important feature promoting migration in lead. No compound containing a single pair of complexable hydroxyl groups shows an appreciable mobility in basic lead acetate.

The mobilities of reducing sugars in sodium hydroxide show a trend towards highest rates for the conformationally least stable sugars, but the relatively high rates for glucose and mannose show that conformational instability is not the sole operative factor. There are indications of a configurational trend in the mobilities of sugar alcohols in sodium hydroxide; those with the *allo* configuration have high mobilities and those with the *ido* configuration, low mobilities, with the others distributed rather irregularly between. The cyclitols have low mobilities in sodium hydroxide, but it may be significant that the conformationally unstable isomers, *muco*-, *allo*-, and *cis*-inositol, are appreciably faster than the conformationally stable isomers. Migration in sodium hydroxide is presumably due to ionization of hydroxyl groups rather than formation of cyclic complexes, and it is difficult to perceive a common stereochemical principle that accounts for the observed wide differences in acidity displayed by stereoisomers in the three groups of compounds. It is surprising to find that some of the heptitols behave as though they are comparable in acidity to reducing sugars, in which the hydroxyl group at the reducing carbon atom is known to be moderately acidic.

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THE CONSTITUENTS OF *CRYPTOCARYA PLEUROSPERMA* WHITE & FRANCIS

I. PLEUROSPERMINE : A NEW ALKALOID OF THE LEAVES

By E. GELLERT*

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Summary

A new alkaloid pleurospermine, $C_{14}H_{19}O_3N$, has been isolated from the leaves of *Cryptocarya pleurosperma*. Pleurospermine contains a methoxyl group, a phenolic hydroxyl group, and a tertiary nitrogen atom. The third oxygen atom is possibly present as an alcoholic hydroxyl group. On heating with palladium-charcoal the alkaloid yields 4-hydroxy-3-methoxyacetophenone (I), while methylation followed by oxidation gives veratric acid.

I. INTRODUCTION

Alkaloids with widely differing structures have been isolated from the bark of several *Cryptocarya* species (Gellert and Riggs 1954). *C. pleurosperma* bark contains the phenanthroquinolizidine alkaloid cryptopleurine (de la Lande 1948) the structure of which has recently been elucidated (Fridrichsons and Mathieson 1954 ; Gellert 1956) and confirmed by synthesis (Bradsher and Berger 1958 ; Marchini and Belleau 1958). The present paper describes the isolation from the leaves of this species of pleurospermine, an alkaloid with a skeletal structure different from that of the bark alkaloid, and the identification of two degradation products of it. The alkaloid content of different samples of leaf varied from 0·006–0·1 per cent.

Pleurospermine has the formula, $C_{14}H_{19}O_3N$, as shown by the analyses of base, hydrochloride, and picrolonate. It is optically active. The presence of one methoxyl group and the absence of carbon-methyl and alkylimide groups were shown by standard analytical procedures. Zerewitinoff estimation established the presence of two active hydrogen atoms. Treatment with nitrous acid gave neither diazonium salt nor nitrosamine, while acetylation, though no crystalline product could be isolated, did not yield a neutral product. In addition the Palumbo (1948) colour reaction, used also in the case of cryptopleurine, was positive thus establishing the tertiary character of the nitrogen atom. Accordingly the two active hydrogen atoms must be tentatively ascribed to the presence of two hydroxyl groups. At least one of the hydroxyl groups is phenolic as shown by its solubility in aqueous sodium hydroxide and by the strong bathochromic

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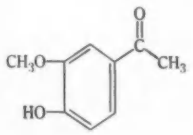
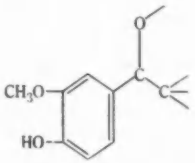
shift (Fig. 1, Table 1) in the ultraviolet absorption maxima in alkaline medium (Morton and Stubbs 1940; Lemon 1947); moreover the alkaloid hydrochloride gives a weak bluish green ferric reaction. The alkaloid did not react with carbonyl reagents. Pleurospermine in glacial acetic acid in the presence of platinum oxide absorbed slightly more than 3 moles of hydrogen but the hydrogenation product was a gum which could not be induced to crystallize. Non-crystallizable gums were also obtained from attempts to benzoylate pleurospermine or to methylate with diazomethane. The uptake of 3 moles of hydrogen, together with the ultraviolet spectrum, indicates the presence in the molecule of an aromatic ring, and consequently, if no carbonyl group is present, of two other rings.

Oxidation of pleurospermine with silver oxide or potassium permanganate gives only a very small amount of oxidation product presumably due to the presence of the phenolic hydroxyl group. However, if first methylated with dimethyl sulphate and the crude reaction mixture then oxidized by permanganate, a small amount of veratric acid is obtained. This acid was identified by direct comparison with an authentic specimen; the infra-red spectrum is recorded in Figure 3. Attempted selenium dehydrogenation of pleurospermine yields only traces of intractable material, but heating pleurospermine hydrochloride with palladium-charcoal yields 4-hydroxy-3-methoxyacetophenone (acetovanillone, I) in approximately 15 per cent. yield. 4-Hydroxy-3-methoxyacetophenone was identified by direct comparison with an authentic specimen prepared as described by Reichstein (1927); the infra-red spectrum is recorded in Figure 3. The ultraviolet spectrum (Fig. 2) is in agreement with that recorded by Lemon (1947), but shows an additional minimum at 215 mμ (Fig. 2, Table 1).



(I)



(II)

The isolation of veratric acid and of 4-hydroxy-3-methoxyacetophenone from the degradation of pleurospermine establishes the partial formula II and suggests, on biogenetic grounds, the probability that the alkaloid is a phenylethylamine derivative. The formation of 4-hydroxy-3-methoxyacetophenone is then consistent with the fission of the C—N bond in such a structure.

There is a striking similarity between the ultraviolet absorption spectrum of the alkaloid and that of 4-hydroxy-3-methoxyacetophenone (Figs. 1 and 2, Table 1) which would suggest, despite the lack of reaction with carbonyl reagents, that pleurospermine is a substituted acetophenone. Support for this is to be found in the infra-red spectra (Fig. 3), pleurospermine absorbing in the carbonyl region at 1660 cm^{-1} essentially the same frequency as does the carbonyl group of 4-hydroxy-3-methoxyacetophenone. However, this interpretation cannot be

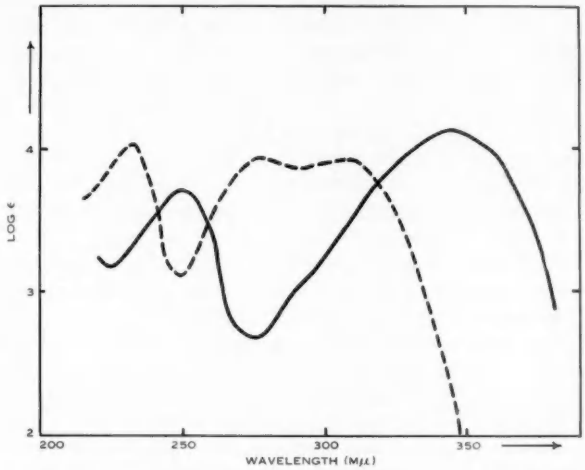


Fig. 1.—Ultraviolet absorption spectra of pleurospermine.
--- Base or hydrochloride in EtOH.
—— In 0·1 per cent. alcoholic KOH.

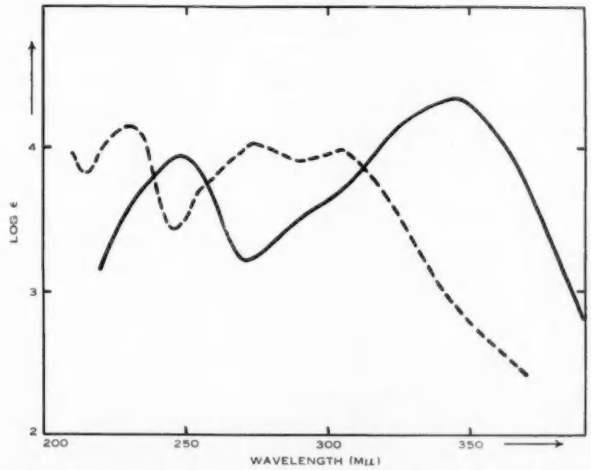


Fig. 2.—Ultraviolet absorption spectra of the 4-hydroxy-3-methoxy-acetophenone, the Pd-C degradation product I of pleurospermine.
--- In EtOH.
—— In 0·1 per cent. alcoholic KOH.

reconciled with the lack of reactivity with carbonyl reagents, with the uptake of three moles of hydrogen nor with the presence of two active hydrogen atoms. Moreover, the observed optical activity is difficult to reconcile with the presence of a carbonyl group and the absence of a C-methyl group. Pending clarification of these points when further plant material becomes available, it seems probable that the alkaloid contains an alcoholic hydroxyl rather than a carbonyl group.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA*

Pleurospermine						Pd-C Degradation Product 4-Hydroxy-3-methoxyacetophenone					
In Alcohol			In Alcoholic KOH			In Alcohol			In Alcoholic KOH		
Max.	Min.	Log ε	Max.	Min.	Log ε	Max.	Min.	Log ε	Max.	Min.	Log ε
	—	—		215	3·18		215	3·83		—	—
233		4·03	245		3·69	230		4·16	248		3·95
	250	3·10		275	2·68		245	3·43		270	3·22
278		3·94	345		4·14	273		4·03	345		4·35
	393	3·86					290	3·92			
310		3·92				305		3·99			

* Measured with a Hilger Uvispek.

II. EXPERIMENTAL

Melting points are corrected. Microanalyses were made by the C.S.I.R.O. Microanalytical Laboratory at the University of Melbourne.

(*a*) *Extraction.*—(i) Milled, dried leaves of *C. pleurosperma* (1·7 kg) were extracted in a Soxhlet with methanol for 42 hr. The extract was evaporated to dryness and the residue warmed repeatedly with dilute acetic acid and then with dilute hydrochloric acid. The combined aqueous acidic solutions (*A*) were extracted with chloroform which yielded on evaporation approx. 18 g of a brown gum (*B*). The solution *A* still gave a faint Mayer's reaction but precipitated no material on basification. It contains traces of two probably quaternary alkaloids (R_F 0·55 and 0·42 in BuOH–AcOH) which were not further investigated. The gum *B* was digested several times with hot dilute acetic acid, and the solution filtered hot, and after cooling overnight filtered again. The aqueous solution was made just alkaline with sodium hydroxide solution and continuously extracted with ether. After evaporation the ether solution yielded c. 6 g of a brown gum (*C*). During this ether extraction a white crystalline solid separated from the aqueous alkaline solution and was filtered off (*D*). After exhaustive ether extraction the aqueous alkaline solution was concentrated and a second crop of crystals, identical with *D*, was obtained. The total yield of these crystals was 1·7 g.

(ii) The ether extract *C* was chromatographed in benzene on alumina. Benzene eluted only a few mg of *crystals*, m.p. 206–207·5 °C. The amount was insufficient for analysis. They are not however identical with *iso*dihydrohomocryptopleurine, m.p. 205–206 °C, as they show 25 °C depression in m.p. on mixing. Resolution of the later fractions was unsuccessful and the material was not investigated further.

(iii) The portion of the gum *B* which was insoluble in dilute acetic acid was chromatographed on alumina in benzene. The oil eluted in the first fraction was distilled at a bath temperature of 145 °C at 0·5 mm/Hg yielding a yellow viscous *liquid* (Found : C, 71·9 ; H, 9·1 ; active H, 0·4% ; mol. wt., 216 (Rast). Calc. for $C_{15}H_{22}O_3$: C, 72·0 ; H, 8·8 ; 1 active H, 0·4% ; mol. wt.,

250). The substance gives a strong brown colour with ferric chloride indicating the presence of an enolic hydroxyl group, and could be similar to ngaiol (Birch, Massy-Westropp, and Wright 1953).

(iv) Extraction of milled, dried leaves (25·8 kg) from a different collection yielded a large amount of gum, but only 1·75 g alkaloid, the isolation of which was more tedious than that described in Section II (*a*) (i).

(*b*) *The Alkaloid.*—(i) *Pleurospermine.* The crude alkaloid *D* from Section II (*a*) (i) was recrystallized several times from water yielding white crystals of m.p. 176–177·5 °C with decomposition (Found: C, 67·2; H, 7·7; O, 19·4; N, 5·7; MeO, 12·2; active H, 0·8% (warm);

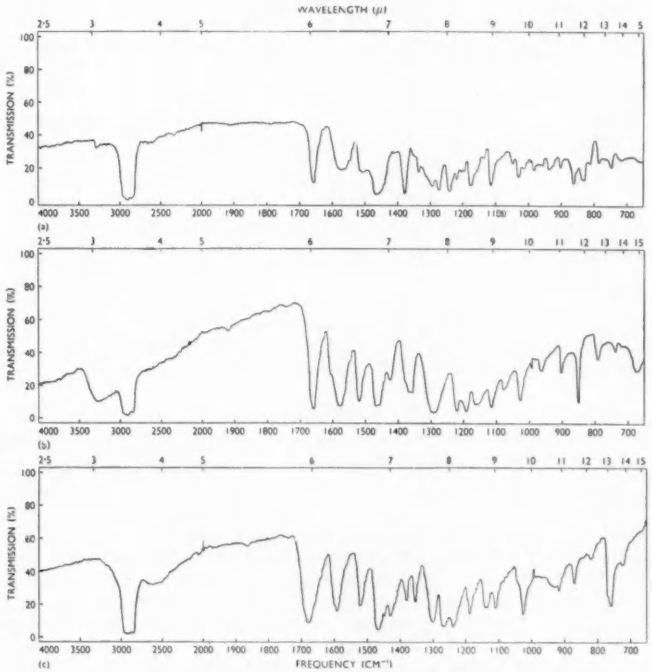


Fig. 3.—Infra-red absorption spectra (in " Nujol mull " at room temperature).
(*a*) Pleurospermine.
(*b*) 4-Hydroxy-3-methoxyacetophenone, the Pd-C degradation product.
(*c*) Veratric acid.

(N)-Me, nil; (C)-Me, nil. Calc. for $C_{14}H_{19}O_3N$: C, 67·4; H, 7·7; O, 19·2; N, 5·6; MeO, 12·4; 2 active H, 0·8%). R_F 0·44 in BuOH–AcOH; $[\alpha]_D^{21}$ +44±2° (*c*, 0·81 in MeOH). The alkaloid is soluble in aqueous sodium hydroxide.

(ii) *Salts.* The *hydrochloride* was prepared from pleurospermine by concentrating a solution in ethanolic hydrochloric acid. After several recrystallizations from ethanol the product melted at 219–220 °C (Found: C, 58·8; H, 7·1; O, 16·8; N, 4·8; Cl, 12·8; MeO, 10·9%. Calc. for $C_{14}H_{19}O_3N.HCl$: C, 58·8; H, 7·0; O, 16·8; N, 4·9; Cl, 12·4; MeO, 10·9%). After neutralizing with Na_2CO_3 solution a concentrated aqueous solution of this hydrochloride, pleurospermine was recovered unchanged and identified by m.p. and mixed m.p. Pleurospermine

hydrochloride gives a weak bluish green colouration with ferric chloride in aqueous solution, which practically disappears on dilution with methanol.

The *picrolonate* was precipitated from a hot aqueous acetic acid solution of pleurospermine by an aqueous solution of picrolonic acid. The compound was then recrystallized several times from aqueous methanol, m.p. 210–212 °C (Found: C, 56·4; H, 5·5; N, 13·7%. Calc. for $C_{14}H_{19}O_2N.C_{10}H_8O_5N_4$: C, 56·1; H, 5·3; N, 13·6%).

The *picrate* was crystallized from aqueous acetone, m.p. 166 °C (decomposition starts at about 150 °C after loss of solvent of crystallization below 100 °C).

(iii) *Derivatives.* Refluxing pleurospermine in ethanolic hydrochloric acid with hydroxylamine or 2,4-dinitrophenylhydrazine or warming pleurospermine with aqueous hydroxylamine hydrochloride in the presence of sodium acetate yields no carbonyl derivatives.

Attempts to acetylate or benzoylate pleurospermine in pyridine with acetic anhydride or benzoyl chloride gave only intractable gums. The acetylated product, after removal of excess of acetic and anhydride and pyridine, was dissolved in ether. When the ethereal solution was shaken with aqueous hydrochloric acid the material was completely extracted by the acid.

Methylation of pleurospermine with diazomethane in methanol also yielded some intractable gummy material.

Pleurospermine at room temperature in glacial acetic acid solution in the presence of PtO_2 absorbs just over 3 moles of hydrogen, but the resulting gum resisted attempts at crystallization.

(c) *Dehydrogenations.*—(i) An attempted selenium dehydrogenation gave only traces of intractable material.

(ii) Pleurospermine hydrochloride (400 mg) was thoroughly mixed with 5% Pd-charcoal (900 mg) and the mixture heated in a sealed tube at 250–260 °C for 15 min. The reaction mixture was then extracted with methanol. After addition of dilute hydrochloric acid to the solution the methanol was evaporated, and the cooled turbid aqueous acidic solution extracted with ether. After drying and evaporating the ether, the residue was sublimed three times, yielding approx. 50 mg of colourless crystals which were recrystallized twice from ether-light petroleum, m.p. 112–114 °C (Found: C, 65·3; H, 6·2; O, 28·6; MeO, 18·5; (C)-Me, 8·5%. Calc. for $C_9H_{10}O_3$: C, 65·1; H, 6·1; O, 28·9; MeO, 18·7; Me, 9·0%). Mixed m.p. with authentic 4-hydroxy-3-methoxyacetophenone (m.p. 113–115 °C) is 112–115 °C. The infra-red spectra are superimposable. The *2,4-dinitrophenylhydrazone*, prepared in the usual way, was recrystallized twice from ethanol, m.p. 245–246 °C (Found: C, 52·2; H, 4·1; O, 27·3; N, 16·1%. Calc. for $C_{15}H_{14}O_6N_4$: C, 52·0; H, 4·1; O, 27·7; N, 16·2%).

(d) *Oxidations.*—(i) Silver oxide or potassium permanganate oxidation of pleurospermine resulted in the isolation of only traces of material, which were not characterized.

(ii) An aqueous alkaline solution of pleurospermine (100 mg) was methylated with dimethyl sulphate on the water-bath, and the crude reaction mixture oxidized with aqueous potassium permanganate. The solution was acidified and extracted with ether. After drying and evaporating the ether, the residue (*c.* 5 mg) was twice recrystallized from water. The substance melts at 180–181 °C, and is identical with veratric acid as shown by mixed m.p. determination and by the identity of their infra-red spectra.

III. Acknowledgments

The author wishes to thank Dr. L. J. Webb and Mr. W. T. Jones for the collection of the plant material, and Mr. A. C. K. Triffett for the measurement of the infra-red absorption spectra.

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CHEMISTRY OF NON-ENZYMIC BROWNING

V. THE PREPARATION OF ALDOSE–POTASSIUM BISULPHITE ADDITION COMPOUNDS AND SOME AMINE DERIVATIVES

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Summary

From the reaction of potassium bisulphite with D-glucose the glucose-potassium bisulphite addition compound, D-*glycero*-D-*ido*-1,2,3,4,5,6-hexahydroxyhexyl potassium sulphonate was prepared pure and crystalline. Similar new compounds were prepared from the reaction of potassium bisulphite with D-galactose, L-rhamnose, D-mannose, and L-arabinose.

The reaction of the glucose-potassium bisulphite addition compound with aniline gave pure crystalline D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl potassium sulphonate. Similar derivatives were prepared from *p*-toluidine, *p*-anisidine, and *o*-phenylenediamine. The aniline and *p*-toluidine derivatives were also prepared from the glucose-sodium bisulphite addition compound.

I. INTRODUCTION

Sulphur dioxide and bisulphites are used in the inhibition of non-enzymic browning in foodstuffs. In such systems reactions involving sugars, amines or amino acids, and bisulphites may occur. Kerp (1904) isolated an impure glucose–sodium bisulphite addition compound by adding alcohol to an aqueous solution of glucose and sodium bisulphite. The pure compound was prepared in an improved method by Braverman (1953). However, the mechanisms by which bisulphites exert their protective action are little understood.

As part of a study of this problem crystalline compounds were prepared from the reaction of aldose sugars with potassium bisulphite. From the glucose–potassium bisulphite addition compound crystalline derivatives were prepared by reaction with amines under suitable conditions.

Reaction of aldoses with potassium bisulphite in place of sodium bisulphite considerably facilitated the isolation of crystalline compounds. D-Glucose and potassium bisulphite in water, for example, reacted at room temperature in 16 hr to yield approximately 50 per cent. of the crystalline addition compound. D-Galactose behaved similarly. With D-mannose an almost quantitative yield of product was obtained in 10 min at room temperature. L-Rhamnose and L-arabinose also gave crystalline addition compounds. The reaction thus appears to be a general one for aldose sugars.

The aldose–bisulphite addition compounds were insoluble in non-polar solvents but soluble in water. The glucose–bisulphite compound dissolved in

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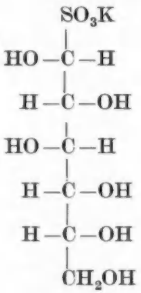
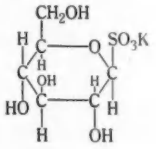
methanol to form a 20 per cent. solution after reflux for 30 min in this solvent. The compound could not be recrystallized from methanol but was recrystallized from methanol containing 10 per cent. water and from water.

The aldose–bisulphite compounds exist either as the acyclic structure I or as the pyranose form (II). A furanose ring form is also possible. Braverman (1953) found that his glucose–sodium bisulphite addition compound analysed correctly for structure I with one molecule of water of crystallization, and one



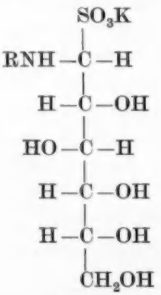
(I)



(III)

molecule of water only was lost by distillation in toluene. He thus suggested I as the more probable structure. The present evidence supports this suggestion. No compound giving an analysis corresponding to II has yet been prepared. The glucose and rhamnose–potassium bisulphite addition compounds analysed correctly for I with one molecule of water of crystallization. Analyses of the compounds from galactose, mannose, and arabinose indicated I without water of crystallization.

The glucose–potassium bisulphite addition compound I is named D-*glycero*-D-*ido*-1,2,3,4,5,6-hexahydroxyhexyl potassium sulphonate (Rules of Carbohydrate Nomenclature 1952, rules 6 and 22). The trivial name glucose potassium sulphonate is also used for this compound.



R = -phenyl ;
-*p*-tolyl ;
-*p*-methoxyphenyl ;
-*o*-aminophenyl ;

(III)

Glucose potassium sulphonate reacted with primary aromatic amines to yield crystalline derivatives III. The aniline derivative of glucose potassium

sulphonate (III; R=-phenyl) is named D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl potassium sulphonate. This system of naming defines the configuration at C1. Evidence supporting the configurations I and III adopted here will be presented in a subsequent paper.

The methods used for preparing the amine derivatives of glucose potassium sulphonate are similar to those used for preparing glycosylamines (Ellis and Honeyman 1955). When glucose potassium sulphonate was heated with excess amine in the presence of a small amount of added water a homogeneous syrup was formed. Addition of methanol or ethanol to this syrup caused crystallization of the product. Alternatively, excess amine was removed by extraction with ether, when the residual syrup crystallized. The amine derivatives were also prepared by refluxing glucose potassium sulphonate in methanol containing the amine. The amine derivatives contained one molecule of water of crystallization. Yields ranged from 60–90 per cent. for the different amines.

Compounds of the type described here have not so far been isolated from foods treated with bisulphite. Indirect evidence for the existence of aldose sulphonates in such foods has been presented (Gehman and Osman 1954). The amine derivatives may play a part in inhibiting those browning reactions (Hodge 1953) which are dependent upon the Amadori rearrangement. Conditions favouring the stability of the aldose sulphonates and their amine derivatives reduce the concentration of glycosylamine and thus the extent to which the Amadori rearrangement can occur.

II. Experimental

Melting points are uncorrected. Microanalyses were made by the C.S.I.R.O. Microanalytical Laboratory at the University of Melbourne.

(*a*) *Reaction of* D-*Glucose and Sodium Bisulphite (according to Braverman 1953).*—D-Glucose (144 g) and sodium metabisulphite (44 g) reacted in water (200 ml), after 7 days at 37 °C, to yield the crystalline product D-*glycero*-D-*ido*-1,2,3,4,5,6-hexahydroxyhexyl sodium sulphonate; 156 g (65%), m.p. 99·0 °C, $[\alpha]_D^{25}$ —3·9° (*c*, 3·8 in 10% acetic acid) (Found: C, 23·4; H, 5·0; S, 10·9; Na, 7·9%. Calc. for $C_6H_{13}O_9SNa.H_2O$: C, 23·8; H, 5·0; S, 10·6; Na, 7·6%). Braverman finds m.p. 92–93 °C, $[\alpha]_D^{20}$ —6° (const. *c*, 10 in hydrochloric acid).

(*b*) *Reaction of Aldoses with Potassium Bisulphite.*—(i) *Reaction of* D-*Glucose and Potassium Bisulphite.* D-Glucose (180 g) dissolved in water (180 ml) was added to a solution of potassium metabisulphite (111 g) in water (120 ml). After standing at room temperature overnight the crystalline product D-glycero-D-ido-*1,2,3,4,5,6-hexahydroxyhexyl potassium sulphonate* (160 g, 52%) was filtered and washed, first with methanol–water (90 : 10 v/v) and finally with methanol. The mother liquor from the filtration was held in an open beaker at 37 °C for 3 days to allow evaporation and further crystallization. These crystals were filtered and washed as described. Recrystallization could be achieved from water or from methanol–water (90 : 10 v/v). After refluxing for ½ hr in the latter solvent the crystals dissolved and the solution was filtered. The product recrystallized after standing 6 days at room temperature. The total yield of crystalline material was 272 g (91%), m.p. 94·0 °C, $[\alpha]_D^{25}$ —4·8° (*c*, 4·1 in 10% acetic acid) (Found: C, 22·7; H, 4·7; S, 10·0; K, 12·3%. Calc. for $C_6H_{13}O_9SK.H_2O$: C, 22·9; H, 4·9; S, 10·0; K, 12·7%). No loss in weight occurred after drying for 1½ hr at 60 °C in a vacuum over P_2O_5.

(ii) *Reaction of* D-*Galactose with Potassium Bisulphite.* D-Galactose (50 g) dissolved in water (50 ml) was mixed with a solution of potassium metabisulphite (30 g) in water (30 ml). After 24 hr at room temperature the crystalline product D-glycero-L-gluco-*1,2,3,4,5,6-hexahydroxyhexyl potassium sulphonate* (42 g, 50%) was filtered and washed as described above. Additional product (32 g, 38%) crystallized when the mother liquor was stood at 37 °C for 3 days. The total yield

of product was 74 g (88%), m.p. 136 °C, $[\alpha]_D^{25}$ 7·7° (*c*, 3·2 in 10% acetic acid) (Found : C, 24·0 ; H, 4·6 ; S, 10·3 ; K, 13·2%. Calc. for $C_6H_{13}O_9SK$: C, 24·0 ; H, 4·3 ; S, 10·1 ; K, 12·6%). No loss in weight occurred when the crystals were dried for ½ hr at 60 °C in a vacuum over P_2O_5.

(iii) *Reaction of* L-*Rhamnose with Potassium Bisulphite.* L-Rhamnose (5 g) in water (5 ml) was mixed with a solution of potassium metabisulphite (3·1 g) in water (5 ml). After standing at 37 °C for 2 days the crystalline product L-galacto-*1,2,3,4,5-pentahydroxyhexyl potassium sulphonate* (4 g, 60%) was filtered and washed, m.p. 97·0 °C, $[\alpha]_D^{25}$ 1·7° (*c*, 2·0 in 10% acetic acid) (Found : C, 24·0 ; H, 5·0 ; S, 10·6 ; K, 13·4%. Calc. for $C_6H_{13}O_9SK.H_2O$: C, 23·8 ; H, 5·0 ; S, 10·6 ; K, 13·0%). No loss in weight occurred on drying for 1 hr at 60 °C in a vacuum over P_2O_5.

(iv) *Reaction of* D-*Mannose with Potassium Bisulphite.* D-Mannose (5 g) dissolved in water (5 ml) was added to a solution of potassium metabisulphite (3·1 g) in water (5 ml). After 10 min at room temperature the crystalline product D-glycero-D-talo-*1,2,3,4,5,6-hexahydroxyhexyl potassium sulphonate* (7·3 g, 88%) was filtered and washed in the usual way, m.p. 149 °C, $[\alpha]_D^{25}$ 7·2° (*c*, 2·6 in 10% acetic acid) (Found : C, 24·0 ; H, 4·5 ; S, 10·4 ; K, 12·9%. Calc. for $C_6H_{13}O_9SK$: C, 24·0 ; H, 4·3 ; S, 10·1 ; K, 12·6%). No loss in weight occurred after drying for ½ hr at 60 °C in a vacuum over P_2O_5.

(v) *Reaction of* L-*Arabinose with Potassium Bisulphite.* L-Arabinose (5 g) dissolved in water (5 ml) was added to a solution of potassium metabisulphite (3·1 g) in water (5 ml). After standing at 37 °C for 5 days the syrup crystallized. The crystals were dissolved in water (2 ml) and the solution seeded. After 2 days at room temperature the product L-*manno-1,2,3,4,5-pentahydroxypentyl potassium sulphonate* (7 g, 77·7%) was filtered and washed. Owing to the viscosity of the syrup adhering to the crystals some precipitation occurred during washing. The product was thus slightly impure, m.p. 78 °C, $[\alpha]_D^{25}$ 13·0° (*c*, 2·8 in 10% acetic acid) (Found : C, 23·0 ; H, 4·6 ; S, 10·5%. Calc. for $C_5H_{11}O_8SK$: C, 22·2 ; H, 4·1 ; S, 11·8%).

(c) *Reaction of Glucose Potassium Sulphonate with Amines.*—(i) *Reaction with Aniline.* (1) Glucose potassium sulphonate (10 g) was heated at 100 °C for ½ hr with excess aniline (8 ml) and water (3 ml) to form a homogeneous syrup. Excess aniline was extracted with ether (two 20-ml portions) after which the syrup crystallized. Washing with ethanol gave the pure white crystalline product D-glycero-D-ido-*1-anilino-2,3,4,5,6-pentahydroxyhexyl potassium sulphonate* (10 g, 80·5%). Addition of methanol (20 ml) to the syrup also gave the crystalline product on standing overnight. The crystals were insoluble in ethanol and methanol, m.p. 142 °C (decomp.), $[\alpha]_D^{25}$ −14·3° (3 min)→+22° (const. 30 min, *c*, 2 in 10% acetic acid) (Found : C, 36·5 ; H, 5·1 ; N, 3·6 ; S, 8·1 ; K, 9·9%. Calc. for $C_{12}H_{18}O_8NSK.H_2O$: C, 37·1 ; H, 4·8 ; N, 3·5 ; S, 8·7 ; K, 9·6%).

(2) Glucose potassium sulphonate (30 g) was refluxed in methanol (150 ml) containing aniline (24 ml). After 1 hr the solution was filtered and the methanol evaporated under vacuum at 40 °C, to small volume. The crystalline product (30 g, 81%) which formed overnight at +1 °C was washed with ethanol. The crystals had the characteristics reported above.

(ii) *Reaction with* p-*Toluidine.* (1) Glucose potassium sulphonate (10 g) was heated with *p*-toluidine (8 g) at 100 °C for ½ hr. No water was required to form a homogeneous syrup. Solution in methanol (20 ml) or extraction of excess base with ether gave the crystalline product D-glycero-D-ido-*1-p-toluidino-2,3,4,5,6-pentahydroxyhexyl potassium sulphonate* (10 g, 78%), m.p. 129 °C (decomp.), $[\alpha]_D^{25}$ +4·0° (3 min)→+22° (const. 30 min, *c*, 2·2 in 10% acetic acid) (Found : C, 38·2 ; H, 5·4 ; N, 3·4 ; S, 7·8 ; K, 10·6%. Calc. for $C_{13}H_{20}O_8NSK.H_2O$: C, 38·0 ; H, 5·4 ; N, 3·4 ; S, 7·8 ; K, 9·5%).

(2) Glucose potassium sulphonate (30 g) was refluxed in methanol (150 ml) containing *p*-toluidine (20 g). After 1 hr the solution was filtered and the methanol evaporated to small volume under vacuum. After 2 days at +1 °C the crystalline product (32 g, 79%) was filtered and washed. The crystals had the characteristics reported above.

(iii) *Reaction with* p-*Anisidine.* Glucose potassium sulphonate (5 g), *p*-anisidine (5 g), and water (1·5 ml) were heated at 100 °C for ½ hr to form a homogeneous syrup. Excess

p-anisidine was extracted with ether (three 20-ml portions). The residual syrup crystallized. The product D-glycero-D-ido-*1*-p-*anisidino-2,3,4,5,6-pentahydroxyhexyl potassium sulphonate* (5·0 g, 72%) was filtered and washed with ethanol; m.p. 126 °C (decomp.), $[\alpha]_D^{25}$ 13·0° (const. *c*, 1·1 in 10% acetic acid) (Found: C, 37·5; H, 5·2; N, 3·5; S, 7·3; K, 10·5%. Calc. for $C_{13}H_{20}O_9NSK.H_2O$: C, 37·0; H, 5·2; N, 3·3; S, 7·2; K, 9·2%).

(iv) *Reaction with* o-*Phenylenediamine.* Glucose potassium sulphonate (10 g) was heated for ½ hr at 100 °C with *o*-phenylenediamine (8 g) and water (3 ml) to form a homogeneous syrup. Excess base was removed with ether when the residual syrup crystallized to give the product, D-glycero-D-ido-*1*-o-*phenylenediamino-2,3,4,5,6-pentahydroxyhexyl potassium sulphonate* (11·5 g, 89%), m.p. 150 °C (decomp.), $[\alpha]_D^{25}$ −5·2° (4 min)→+12·5° (const. 40 min, *c*, 2·2 in 10% acetic acid) (Found: C, 35·9; H, 5·1; N, 6·5; S, 7·5; K, 9·1%. Calc. for $C_{12}H_{19}O_8N_2SK.H_2O$: C, 35·3; H, 5·1; N, 6·9; S, 7·8; K, 9·6%).

(*d*) *Reaction of Glucose Sodium Sulphonate with Amines.*—(i) *Reaction with Aniline.* Glucose sodium sulphonate (10 g) was heated with aniline (8 ml) and water (3 ml) at 100 °C for ½ hr to form a homogeneous syrup. Excess amine was removed with ether when the residual syrup slowly crystallized. The product D-glycero-D-ido-*1-anilino-2,3,4,5,6-pentahydroxyhexyl sodium sulphonate* (8·0 g, 64%) was washed with ethanol, m.p. 115 °C (decomp.), $[\alpha]_D^{25}$ −6·3° (4 min)→+23° (const. 30 min, *c*, 2 in 10% acetic acid) (Found: C, 38·0; H, 5·5; N, 3·6; S, 8·4; Na, 6·3%. Calc. for $C_{12}H_{18}O_8NSNa.H_2O$: C, 38·0; H, 5·3; N, 3·7; S, 8·5; Na, 6·1%).

(ii) *Reaction with* p-*Toluidine.* Glucose sodium sulphonate (10 g) was heated with *p*-toluidine (8 g) and water (3 ml) as in the method above. The crystalline product D-glycero-D-ido-*1*-p-*toluidino-2,3,4,5,6-pentahydroxyhexyl sodium sulphonate* (7·2 g, 58%) was washed with ethanol m.p. 92 °C (decomp.), $[\alpha]_D^{25}$ −1·1° (4 min)→+22° (const. 30 min, *c*, 2 in 10% acetic acid) (Found: C, 39·7; H, 5·6; N, 3·5; S, 8·2; Na, 5·9%. Calc. for $C_{13}H_{20}O_8NSNa.H_2O$: C, 39·6; H, 6·1; N, 2·8; S, 8·7; Na, 6·7%).

III. Acknowledgment

The author is indebted to Dr. T. M. Reynolds for helpful discussion.

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SHORT COMMUNICATIONS

CALCULATION OF DIFFUSION COEFFICIENTS WITH THE CONTINUAL MONITORING CAPILLARY METHOD*

By R. Mills† and E. W. Godbole†

A recent paper by Mills and Godbole (1958) described a precise method of determining single ion diffusion coefficients by the continual monitoring of radioactivity in open-ended capillaries. In that paper, the treatment of data to obtain diffusion coefficients, involved measurement of c_0, the radioactivity content of the capillary before the commencement of diffusion. However, such determinations are accompanied by appreciable error which stems essentially from two sources. The first and more serious of these arises from the difficulty in filling the capillary very precisely to its upper boundary, then sealing it effectively, for immersion in the outer solution. Replicate determinations of c_0 values seldom have a precision better than $\pm 0{\cdot}4$ per cent. The continual exposure of the photomultiplier tube and scintillator to light during these operations is probably a contributory cause of this imprecision. The second source of error may arise from the necessity to apply an appreciable " gradient correction " to the $c_{av.}$ figures. As explained in the above paper, this correction is necessary so that the activity measured after diffusion, when a gradient exists, can be compared to that prior to diffusion, when it is uniformly distributed.

A method has now been developed in which ion-diffusion coefficients can be obtained solely from repeated measurements of $c_{av.}$. By this device, errors arising from the procedures detailed in the last paragraph can be eliminated.

Method of Calculation

The diffusion equation used in this treatment is the abbreviated form suitable for long diffusion times (Wahl and Bonner 1951):

$$\pi^2 Dt/4l^2 = \ln (8/\pi^2 \times c_0/c_{av.}). \quad \ldots\ldots\ldots\ldots \quad (1)$$

This equation can be expressed in the form

$$(\pi^2 D/4l^2)t_1 + \ln c_{av.1} = \ln 8c_0/\pi^2, \quad \ldots\ldots\ldots\ldots \quad (2)$$

where t_1 refers to a particular period of diffusion and $c_{av.1}$ the residual activity at the end of such a period.

A plot is now made of $\ln c_{av.}$ against t and the slope of the best straight line through these points gives the value of $\pi^2 D/4l^2$ whence D can be calculated. Alternatively, a least squaring procedure can be used to calculate the slope

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directly. It should be pointed out that in the previous procedure in which $\ln c_0/c_{av.}$ was plotted against time, the experimental points were least squared in relation to a fixed pivoting point. The position of this intercept is in effect a function of c_0 and thus any error in the measured value of the latter would be reflected in the slope of the line. In the present method the slope of the line is dependent only on the precision of the $c_{av.}$ measurements. It has been found experimentally that the best precision is obtained if the measurements are made after equally spaced intervals of a few hours.

Gradient Correction

In our previous paper (Mills and Godbole 1958) the "gradient correction" allowed comparison of $c_{av.}$, the activity gradient measurement with c_0 the initial, uniformly distributed activity. In the amended procedure described above, however, any corrections necessary are those among a series of activity gradients only. Calculations have been made of the relative efficiency of counting the gradients present after known periods of diffusion, and suitably normalizing them by use of the correction factor

$$\int_0^l c(x)\bar{c}(x)\mathrm{d}x \Big/ \int_0^l c(x)\mathrm{d}x \cdot \int_0^l \bar{c}(x)\mathrm{d}x.$$

This expression represents the factor by which the measured activity at any time would have to be multiplied in order to compare it with a uniform distribution of the same total activity. In Table 1, these factors have been calculated for Na^+ ion diffusing in 1·44M aqueous sodium chloride solutions at 25 °C using capillary tubes 2 cm in length. For this system $D = 1{\cdot}202 \times 10^{-5}$ cm²/sec (Mills 1955). The scintillation crystal had a diameter of 3·8 cm and a height of 2 cm.

TABLE 1

Diffusion Period (hr)	Correction Factor for Uniformity	Correction Factor for Gradient
24	1·0096	1·0001
36	1·0094	1·0003
48	1·0097	1·0000
60	1·0097	1·0000
72	1·0093	1·0004

It will be observed in this instance that although a correction of 1 per cent. is necessary to compare the activity gradients with a uniform distribution, the intercomparison of the former alone requires corrections much less than 0·1 per cent. Corrections of this order are normally well below the statistical counting error and can therefore be safely neglected.

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HEATS OF SOLUTION FROM VAPOUR–LIQUID PARTITION CHROMATOGRAPHY*

By B. H. Pyke† and E. S. Swinbourne‡

The separation of benzene, *cyclo*hexane, and *cyclo*hexene by means of vapour-liquid partition chromatography was reported by Anderson and Napier (1956), who used a polyethylene-glycol-octyl-cresyl ether (PGCE) as a stationary phase. The heats of solution of benzene and *cyclo*hexane vapour in PGCE liquid were also determined with this technique (Anderson and Napier 1957).

In a similar study we recently estimated the heats of solution of benzene, *cyclo*hexene, *cyclo*hexane, and *cyclo*pentene vapours in PGCE, the values together with those of Anderson and Napier are presented in Table 1. Taking into consideration possible variation in technique and in quality of the stationary phase§

Table 1

Heats of Solution of Hydrocarbon Vapours in PGCE Liquid

Hydrocarbon Vapour	Heat of Solution (ΔH)		Latent Heat of Condensation (ΔH) (kcal/mole)
	Anderson and Napier (kcal/mole)	Pyke and Swinbourne (kcal/mole)	
Benzene ..	—7·5	—7·2	—7·5*
*cyclo*Hexene ..	Not determined	—7·0	—7·3*
*cyclo*Hexane ..	—5·3	—5·5	—7·2*
*cyclo*Pentene ..	Not determined	—5·1	—6·7†

* Values from Lange (1956).

† Estimated from Trouton's constants of like compounds, using the values obtained for the other compounds from Lange (1956).

there is good agreement in the case of the two substances studied commonly. Further, it is interesting to note that the heats of solution of benzene and *cyclo*hexene are very close in magnitude to the corresponding values for the latent heat of condensation (also shown in Table 1). This comparison indicates that there is no great departure from ideal solution behaviour in these compounds

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§ PGCE is used as a commercial detergent.

(see, however, discussion by Anderson and Napier 1956, p. 543). On the other hand, it appears likely that *cyclo*hexane and *cyclo*pentene are exhibiting *positive* deviation from the ideal vapour pressure/composition law; this would account for *cyclo*hexane appearing in the eluate well in advance of benzene in spite of the higher boiling point of the former (benzene, b.p. 80·1 °C/760 mm; *cyclo*hexane, b.p. 81·4 °C/760 mm).

The apparatus used in the present study was based upon that described by Ray (1954). Alumina-free refractory brick,* carefully ground and screened to −50 to +100 B.S.S. mesh, was used as a support for the stationary phase,

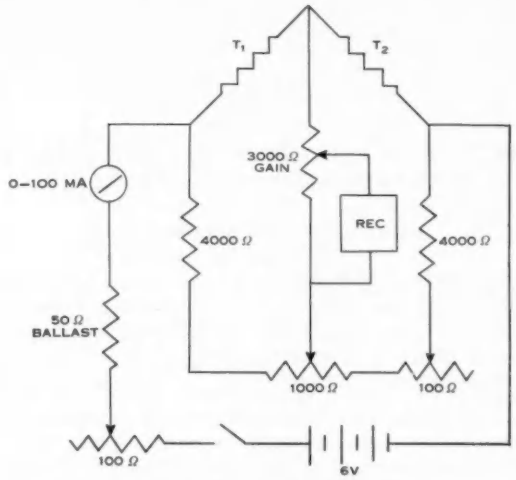

Fig. 1.—The detector circuit.
T_1, T_2, thermistors (resistance 2000 Ω at 20 °C); *Rec*, Leeds and Northrup Speedomax recorder (0–5 mV).

the carrier gas being dry nitrogen. Chromatographic runs were made at temperatures of 62·5, 80, and 100 °C. The thermal conductivity cell was operated at room temperature, thermistors being used as the sensitive elements. Full details of the detector circuit are shown in Figure 1. (Forss and Stark (1958) have recently described similar circuits incorporating the same type of thermistor.) A more comprehensive discussion of the use of thermistors in thermal conductivity gas analysers has been given by Walker and Westenberg (1957).

The authors acknowledge with thanks a gift of polyethylene-glycol-octylcresyl ether from Messrs. Robert Bryce & Co. Ltd., Sydney; and are also grateful to Standard Telephones and Cables Pty. Ltd., Sydney, for assistance in selecting thermistor types.

* Bricks of the " Morgan M.I. 23 and 28 " type were originally used but owing to their high-alumina content were found to be unsatisfactory. Subsequently, the material used was a crushed " Diatom " brick manufactured by Messrs. Newbold General Refractories Ltd.

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ATTEMPTS TO PREPARE THE PHTHALOYL DERIVATIVE OF TETRAKISAMINOMETHYLMETHANE*

By J. A. LAMBERTON†

Although the tetra-*p*-toluenesulphonyl and other derivatives of tetrakisaminomethylmethane (I) have been successfully prepared (van Alphen 1938; Litherland and Mann 1938) consideration of molecular models makes the formation of a tetraphthaloyl derivative seem unlikely on steric grounds. Two methods have been tried for the preparation of this tetraphthaloyl derivative.

When a solution of pentaerythrityl tetrabromide or tetraiodide, phthalimide, and potassium carbonate in dimethylformamide is heated under the conditions of the modified Gabriel synthesis (Billmann and Cash 1953), carbon dioxide and ammonia are evolved and only a low yield of a diphthalimido compound, $C_{29}H_{14}O_4N_2$, is obtained. As pentaerythrityl tetrabromide may be regarded as a substituted *neo*pentyl compound (Dostrovsky, Hughes, and Ingold 1946) it is not surprising that the reaction in this instance should be accompanied by rearrangement, but the method seemed worth attempting because Litherland and Mann obtained the tetra-*p*-toluene sulphonyl derivative of I by heating pentaerythrityl tetrabromide with the sodium salt of *p*-toluene sulphonamide.

To explain the analytical results a formula such as II was considered for the resulting diphthalimido compound because of the observation by Issidorides and Mator (1955) that pentaerythrityl monobromide under alkaline conditions yields some 2-methylene-1,3-propane diol with the elimination of formaldehyde. This must be excluded because the diphthalimido compound shows no unsaturation and a cyclic structure is probable.

When the tetramine (I), prepared by the method of Litherland and Mann, is fused with phthalic anhydride or heated with phthalic anhydride and sodium

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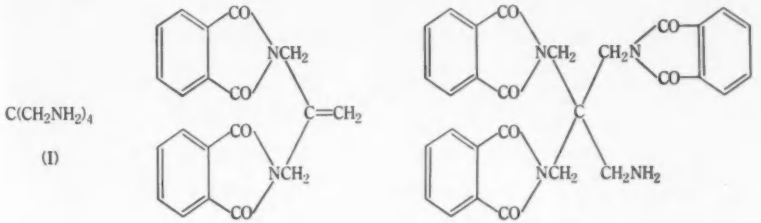
acetate in acetic acid, the triphthaloyl compound (III) is obtained. Evidently steric effects prevent introduction of a fourth group, but the free-amino group, although shielded by the phthalimido groups, still possesses salt-forming properties.



Experimental

Melting points are corrected. Microanalyses were carried out in the C.S.I.R.O. Microanalytical Laboratory at the University of Melbourne.

(*a*) *Diphthalimido Compound.*—In a typical experiment pentaerythrityl tetraiodide (5·9 g), phthalimide (6·1 g), anhydrous potassium carbonate (3·6 g), and dimethylformamide (30 ml) were heated at 140 °C for 7 hr. The reaction mixture was poured into water and the sticky mass which separated was washed several times by stirring with water and with 1% aqueous sodium hydroxide solution to remove phthalimide. After warming with ethanol (30 ml) the crude product (1·0–1·1 g) was filtered and crystallized several times from glacial acetic acid. The purified compound (0·6–0·7 g) was obtained as colourless needles, m.p. 224–225 °C. The same product was obtained when pentaerythrityl tetrabromide was used. During the course of the reaction both ammonia and carbon dioxide were evolved and ammonium carbonate deposited in the condenser. The yield of the compound, m.p. 224–225 °C, was never high, and was approximately the same whether the reaction mixture was heated for 2–3 hr or for 20 hr, but with the shorter period of heating it had to be separated from unreacted iodo compound. A lower reaction temperature (e.g. 100 °C for 8 hr) gave much unreacted tetraiodide (Found : C, 69·2 ; H, 4·0 ; N, 8·4% after repeated crystallization from acetic acid. The same material after further crystallization from chloroform–ethanol gave C, 69·1 ; H, 4·2 ; N, 8·4% ; mol. wt., 330 (Rast) and 343 (Signer). Calc. for $C_{20}H_{14}O_4N_2$: C, 69·4 ; H, 4·1 ; N, 8·1% ; mol. wt., 346). With hydrazine in methanol this compound readily gave phthaloylhydrazine and a base which was converted into a *p*-toluenesulphonyl derivative, m.p. 170 °C. The infra-red absorption spectrum of the diphthalimido compound had strong bands at 1715 and 1774 cm^{-1}, and strongly resembled the infra-red spectrum of 1,4-diphthalimido butane. There were no bands at 3080 or 1645 cm^{-1} where an ethylenic bond such as that in II would be expected to absorb. The compound was recovered unchanged after attempted hydrogenation over platinum oxide in acetic acid containing perchloric acid, and only slowly decolourized a solution of bromine in carbon tetrachloride.

(*b*) *Triphthaloyl Compound (III).*—A sample of tetrakisaminomethylmethane prepared by the method of Litherland and Mann was fused with excess of phthalic anhydride. The fusion mixture was heated with ethanol and the insoluble portion crystallized from aqueous acetic acid. This gave the triphthaloyl derivative (III) as colourless needles, m.p. 284–285 °C, which were readily soluble in glacial acetic acid and dilute hydrochloric acid (Found : C, 66·9 ; H, 4·5 ; N, 10·7%. Calc. for $C_{23}H_{22}O_6N_4$: C, 66·7 ; H, 4·2 ; N, 10·7%). The same compound, m.p. 284–285 °C, was obtained by heating the tetramine with phthalic anhydride and fused sodium acetate in glacial acetic acid.

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CORRIGENDUM

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Page 263, line 11 from the bottom should read: The filter which gave minimum transmission at 50 c/s introduced a resistance of 600,000 Ω in series with the input, ...

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